

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
12 December 2002 (12.12.2002)

PCT

(10) International Publication Number
WO 02/099111 A2

(51) International Patent Classification⁷: C12N 15/82

(21) International Application Number: PCT/CA02/00834

(22) International Filing Date: 7 June 2002 (07.06.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/296,159 7 June 2001 (07.06.2001) US

(71) Applicant (for all designated States except US): NATIONAL RESEARCH COUNCIL OF CANADA [CA/CA]; 1200 Montreal Road, Ottawa, Ontario K1A 0R6 (CA).

(72) Inventors; and

(75) Inventors/Applicants (for US only): SELVARAJ, Gopalan [CA/CA]; 540 Nesslin Crescent, Saskatoon,

Saskatchewan S7J 4V5 (CA). WANG, Aiming [CN/CA]; 114 Brunst Crescent, Saskatoon, Saskatchewan S7N 3S3 (CA). XIA, Qun [CA/CA]; 10-8 Bateman Crescent, Saskatoon, Saskatchewan S7H 3C2 (CA). XIE, Wenshuang [CA/CA]; 4-2720 Main Street, Saskatoon, Saskatchewan S7H 0M3 (CA).

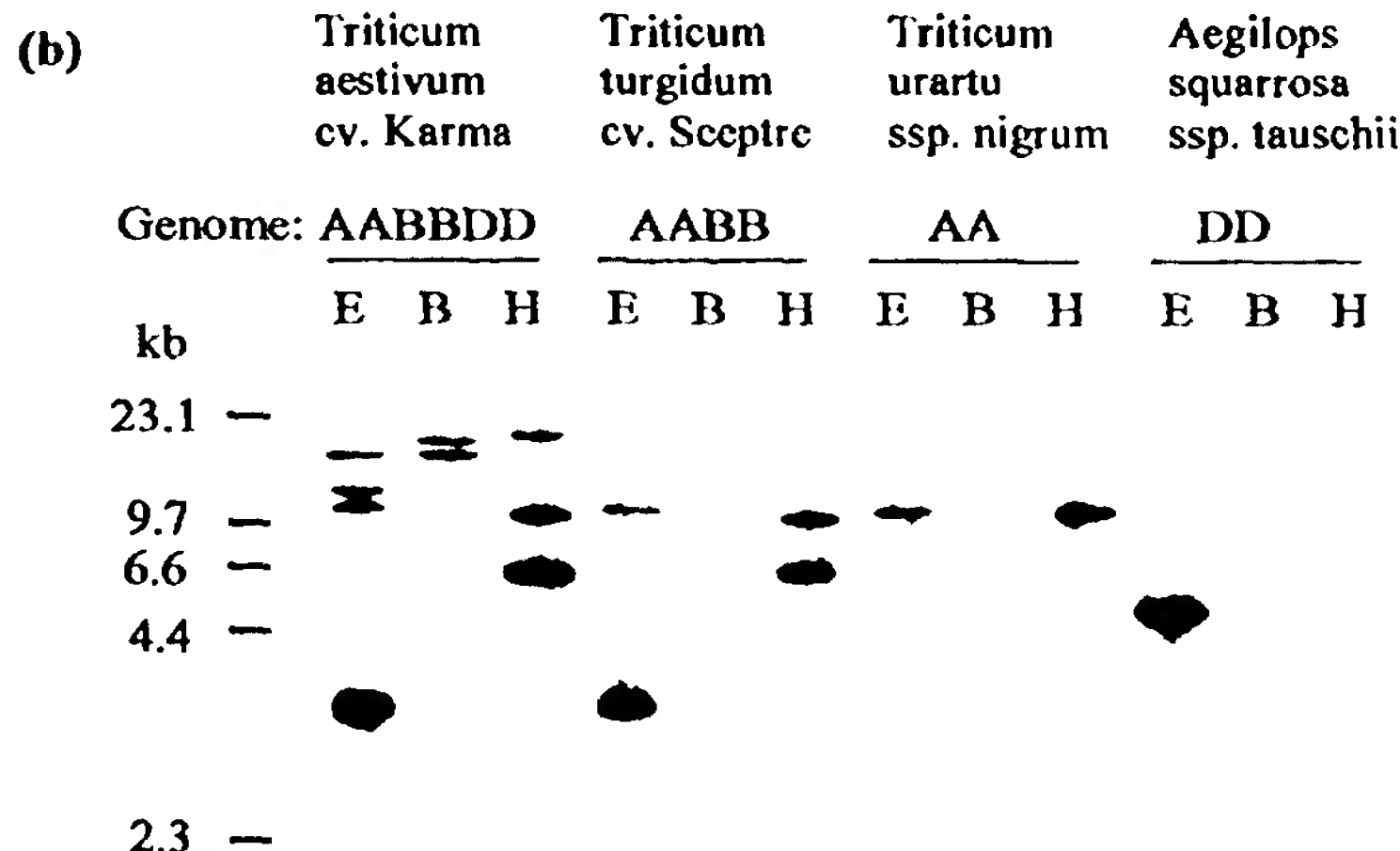
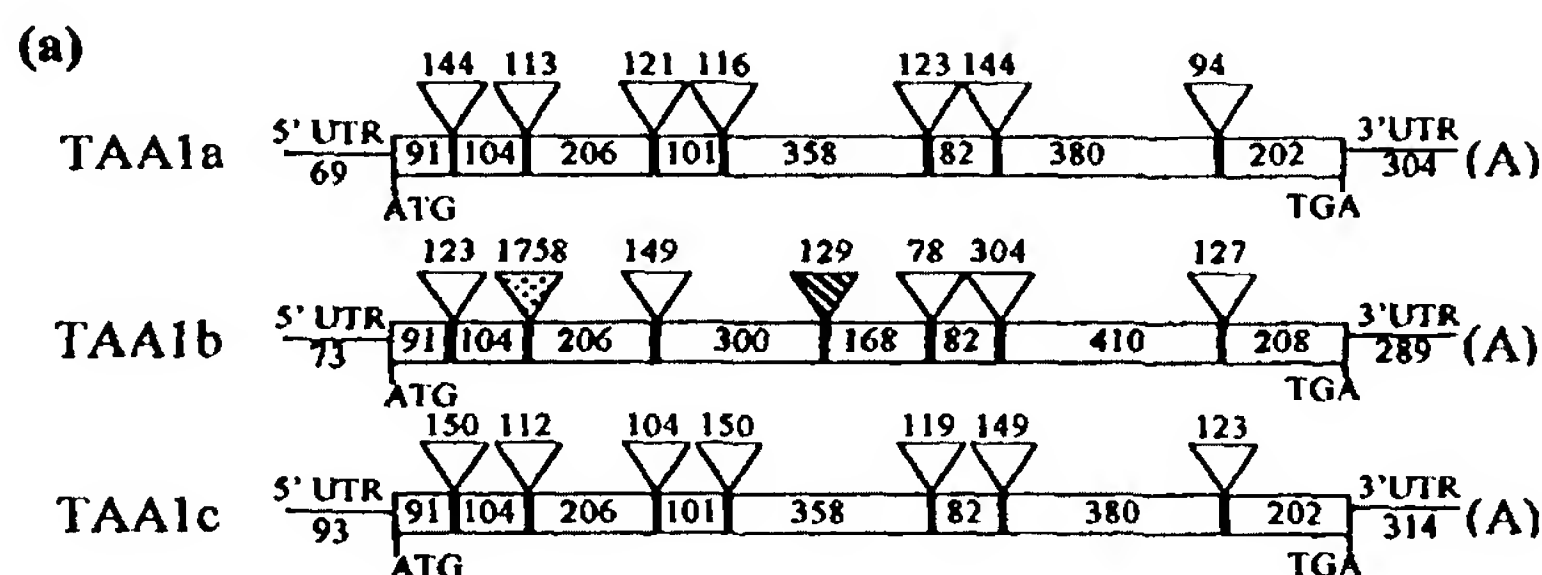
(74) Agents: GALE, Edwin, J. et al.; Kirby Eades Gale Baker, Box 3432, Station D, Ottawa, Ontario K1P 6N9 (CA).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),

[Continued on next page]

(54) Title: ANTHR-SPECIFIC TAA1 GENES ENCODING FATTY ACYL CO-A REDUCTASES, AND USES THEREOF



(57) Abstract: The present invention provides isolated and purified polynucleotide sequences encoding fatty acyl Co-A reductase (FAR) enzymes derived from wheat, designated *TAA1* genes. The invention encompasses genes that encode FAR enzymes that are useful in the production of transgenic plants and other organisms that comprise increased or otherwise altered levels of fatty alcohols. Such plants may have significant commercial value for the production of fatty alcohols for use in nutritional and pharmaceutical compositions. The invention also provides corresponding *TAA1* anther-specific promoters, suitable for the expression of proteins other than FAR enzymes in the anthers and pollen cells of suitably transformed plants.



Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,
GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG).

— with (an) indication(s) in relation to deposited biological
material furnished under Rule 13bis separately from the
description

Published:

— without international search report and to be republished
upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

ANTHER-SPECIFIC TAA1 GENES ENCODING FATTY ACYL CO-A
REDUCTASES, AND USES THEREOF

1. FILED OF THE INVENTION

5

The present invention relates to genes that are specifically expressed in the anthers of plants. More particularly, the present invention relates to genes encoding fatty acyl Co-A reductase enzymes that are required for pollen grain maturation.

10

2. BACKGROUND TO THE INVENTION

There is a significant degree of commercial interest in the development of transgenic plants with altered lipid metabolism, which generate altered or increased yields of lipid products. The development of such modified plants and crops may facilitate the manufacture of nutritional and medicinal products in crops. Therefore, the possibility of successfully generating lipid-modified plants has implications for both the agricultural and pharmaceutical industries.

20 The metabolic pathways that regulate lipid metabolism in plants are not fully understood. Different regions and organs of a plant generate alternative profiles of lipid products, with certain regions of a plant comprising a greater concentration of lipid products than others. For this reason, the genes involved in lipid metabolism must undergo differential regulation for specific lipid products to be concentrated in particular regions of the plant. Delineation of plant lipid metabolic pathways, and the generation of modified transgenic plants with beneficial characteristics, represents a considerable challenge to those of skill in the art.

30 The outer surface of pollen grains represents one region of a plant known to harbor higher concentrations of lipid products. The anthers of plants have

evolved to coat pollen grains with an oily substance to preserve and increase the viability of the pollen. For this purpose, male gametophyte development, and in particular the interplay between the sporophytic tapetum and gametophytic microspore, is a well-orchestrated process in plants (Goldberg et al., 1993). To date, a number of genes underlying this process have been isolated and characterized. These genes may be grouped into pollen-specific and anther/tapetum-specific genes. The former are usually predominantly expressed during advanced stages of pollen development. The examples include genes encoding cytoskeletal proteins (Kandasamy et al., 1999; Lopez et al., 1996), cell wall-degrading enzymes (Brown and Crouch, 1990; Futamura et al., 2000), pollen allergens (Rafnar et al., 1991) and other genes with unknown functions (Zou et al., 1994). The other group includes genes preferentially expressed in the tapetum at relatively early stages of microsporogenesis. These include genes associated with programmed cell death (Walden et al., 1999), pollen excise formation (Aarts et al., 1997; Fuerstenberg et al., 2000; Koltunow et al., 1990), lipid transfer (Aguirre and Smith, 1993), cell wall-degradation (Bih et al., 1999; Hird et al., 1993; Rubinelli et al., 1998) and unknown functions (Jeon et al., 1999).

The anther tapetum plays a pivotal role in plant gametophyte development (Piffanelli et al., 1998; Shivanna et al., 1997). In addition to breakdown of callus wall around microspore tetrads and supply of nutrients to developing pollens, the essential function of the tapetum is thought to form two extracellular lipid-derived structures (pollen exine and pollen coating) of pollen grains. This assumption is established on the base of the earlier cytological observations, and recent ultrastructural and molecular studies (for recent reviews see Furness and Rudall, 2001; Huysmans et al., 1998; Piffanelli et al., 1998). For example, it has been shown that during the development of the extracellular lipidic structures, the tapetum and not the microspore is the major site of fatty acid biosynthesis (Piffanelli et al., 1997). Mutation of a tapetum-specific gene encoding a putative

fatty lipid reductase leads to formation of exine-free pollen and male sterility (Aarts et al., 1997). Recent progress also includes the finding that two tapetum-unique lipidic organelles whose major constituents are neutral esters and polar lipids, upon lysis of the tapetal cells, are discharged into the anther locule and
5 their components contribute to the formation of the lipidic coating of mature pollen grains (Hernández-Pinzón et al., 1999; Piffanelli and Murphy, 1998; Ting et al., 1998; Wu et al., 1997).

These findings substantially facilitate our understanding of the intrinsic link
10 between the tapetal lipid biosynthesis and microspore development. However, the enzymes that catalyze and regulate the biochemical production of these tapetal lipidic compounds have remained unclear. Isolation and characterization of these lipid-specific enzymes would permit an improved understanding of the mechanisms of plant lipid metabolism.

15

3. SUMMARY OF THE INVENTION

It is an object of the present invention to isolate and characterize anther-specific genes involved in lipid metabolism, for the commercial development of useful
20 transgenic plants and plant products.

It is a further object of the present invention to provide a means for modifying lipid metabolism in plants, preferably by increasing or altering the yield of useful lipid-based products in the plants. The present invention further aims to provide a
25 transgenic plant with increased levels of fatty alcohols, which can be harvested for use in the production of, for example, nutritional and pharmaceutical products.

It is another object of the present invention to provide a means of increasing the
30 levels of fatty alcohols in designated regions or organs of a plant, for specific

commercial purposes. These commercial purposes may include, but are not limited to, the production of crops with increased pesticide resistance, crops with altered cross-breeding activity, plants with increased levels of lipid products concentrated in regions that permit facile harvesting and extraction.

5

It is further an object of the present invention to isolate and characterize genes expressed in the tapetum, and their corresponding proteins, that are required for the formation of the outer cell wall of pollen grains during microspore development. In this way, the present invention aims to alter anther-specific properties of a plant to induce, for example, male sterility, and developmental or reproductive modifications that are commercially useful properties. It is still further an object of the present invention to provide a transgenic plant, comprising a construct wherein anther-specific promoters are utilized to generate useful products in the anthers and pollen cells of the transgenic plants.

15

It is a further object of the present invention to provide isolated recombinant proteins involved in plant lipid metabolism, which can be used in the commercial ex vivo production of fatty alcohols.

20 The peptides of the present invention, and their corresponding nucleotide sequences, have significant potential for use in the generation of genetically modified plants with altered profiles, or increased or otherwise altered levels of lipid compounds, as well as plant having desirable anther-specific and whole-plant phenotypic modifications.

25

The inventors of the present application have succeeded in isolating and purifying both the genomic and cDNA sequences of a family of three closely related genes that are predominantly expressed in the tapetum of anthers. The genes were isolated from the bread wheat species '*Triticum aestivum*', and are designated

30 *TAA1a*, *TAA1b*, and *TAA1c*. The cDNA sequences of these genes have

permitted the characterization of the corresponding protein products, which can function as fatty acyl Co-A reductases. Transgenic plants overexpressing a *TAA1* gene can comprise higher than normal levels of fatty alcohols. It is considered that similar transgenic plants will have strong potential for the generation of crops capable of producing fatty acid products for agricultural, nutritional and pharmaceutical purposes.

Moreover, isolation of the corresponding genomic DNA sequences for the *TAA1* genes has permitted the characterization of the anther-specific *TAA1* gene promoters. These promoters are tapetum specific and have significant potential for the generation of constructs for use in transgenic plants, wherein the constructs comprise a gene of choice under the control of the anther-specific promoter. In this way, numerous properties of the plant can be modified to alter, for example, the developmental, reproductive, and aesthetic properties of the plant.

In accordance with a first embodiment of the present invention, there is provided an isolated and purified nucleotide sequence, characterized in that the nucleotide sequence is endogenously expressed in wheat anthers, and encodes a peptide having fatty acyl Co-A reductase (FAR) activity. The present invention therefore provides characterization of a novel family of genes that are involved in lipid metabolism in anthers of plants and encompasses all such corresponding homologous genes.

In an alternative embodiment the present invention provides an isolated and purified nucleotide sequence, characterized in that the nucleotide sequence is selected from:

- (a) a *TAA1* gene, or a part thereof, or a complement thereof; and
- (b) a nucleotide sequence having at least 50% identity to a peptide encoded by a *TAA1* gene, or a part thereof, or a complement thereof;

the nucleotide sequence encoding a protein or a part thereof, that alters lipid metabolism in a transgenic plant exogenously expressing said nucleotide sequence.

5 Preferably, the isolated and purified nucleotide sequence is selected from:

(a) SEQ ID NO: 1, 3, or 5, or a part thereof, or a complement thereof;

and

(b) a nucleotide sequence having at least 50% identity to a peptide encoded by a SEQ ID NO: 1, 3, or 5, or a part thereof, or a complement thereof;

10 the nucleotide sequence encoding a protein or a part thereof, that alters lipid metabolism in a transgenic plant exogenously expressing said nucleotide sequence. More preferably, the nucleotide sequence has at least 70%, more preferably 90%, more preferably 95% and most preferably 99% identity to a peptide encoded by a SEQ ID NO: 1, 3, or 5, or a part thereof, or a complement
15 thereof. In this way, the nucleotide sequences of the present invention include *TAA1* homologous genes from species of plants other than wheat, as well as closely related wheat homologues, polymorphisms and mutated variants of the genes. The invention further encompasses nucleotide sequences that will bind to SEQ ID NOS: 1, 3, or, 5 under stringent hybridization conditions, including
20 nucleotide sequences suitable for use as hybridization probes, PCR primers and DNA sequencing primers.

In further embodiments, the present invention also encompasses isolated and purified peptides, or parts thereof, encoded by *TAA1* genes, or possible variants
25 of the *TAA1* genes disclosed herein. Such peptides may be used in the production of pharmaceutical or nutritional agents as appropriate.

The present invention further encompasses expression cassettes and constructs comprising *TAA1* gene sequences and variants, complements, or parts thereof.

30 Preferably, the expression cassettes and constructs include a *TAA1* gene sequence

open reading frame operably linked to a promoter for expression of the *TAA1* gene product, or part or variant thereof. Preferably, the expression cassettes and constructs of the present invention are suitable for transformation into plants. In this way, transgenic plants having altered lipid metabolism or altered lipid
5 content can be generated. More preferably, the altered lipid metabolism or altered lipid content at least partly occurs within the anthers and / or pollen of the transgenic plant.

The transgenic plants of the present invention therefore include plants expressing
10 the nucleotide sequences disclosed herein, and homologues and variants thereof, thereby increasing, decreasing or changing the lipid content of the plant compared to an unmodified plant. More preferably, the change in lipid content may specifically relate to the fatty alcohol content of the plant, and more preferably the fatty alcohol content of the anthers and / or pollen of the plant.

15 The transgenic plants of the present invention include species of a woody plants, non-woody plants, and grasses, as well as plants selected from the group consisting of crucifer crops, tobacco, wheat, corn, sugar cane, and apple.

In an alternative embodiment, the transgenic plants of the present invention may
20 include constructs wherein the *TAA1* gene or part or variant thereof is under the control of an organ-specific promoter. In this way, the promoter can direct the expression of the nucleotide sequence to affect a particular organ or organs of the plant. The transgenic plants of the present invention may exhibit one or more modified characteristics compared to an unmodified plant including, but not
25 limited to: increased pest resistance; male sterility; reduced height; reduced internode spacing; increased resistance to wind damage; reduced growth rate; altered cross-pollination specification; increased fruit or nut aesthetic appeal; delayed vegetative development; and delayed propagative development.

The transgenic plants of the present invention may contain constructs characterized in that the nucleotide sequence expressed is oriented for antisense expression from the construct, thereby causing a reduction in the levels of fatty acyl Co-A reductase compared to an unmodified plant, and a corresponding
5 decrease in the levels of fatty alcohols present in the plant.

The present invention further encompasses an isolated and purified nucleotide sequence, characterized in that the nucleotide sequence is selected from:

- (a) SEQ ID NO: 7, 8, or 9 or a complement thereof; and
- 10 (b) a nucleotide sequence that can hybridize to SEQ ID NO: 7, 8, or 9 or a complement thereof under stringent hybridization conditions. Therefore, the invention encompasses the corresponding genomic DNA sequences for the *TAA1* family of genes, including promoter sequence disclosed in SEQ ID NOS: 7, 8, and 9, or *TAA1* promoter sequence obtained by chromosome walking a genomic
15 DNA library for 5' (and 3') untranslated regions of the *TAA1* genomic DNAs. Furthermore, in alternative embodiments the invention includes nucleotide sequences for use as hybridization probes, PCR primers or DNA sequencing primers, that bind to the *TAA1* sequences under stringent hybridization conditions. Preferably, the promoters of the present invention can be used to
20 direct the expression of a gene unrelated to fatty acyl Co-A reductases in the anthers and pollen grains of transgenic plants. Most preferably, the promoter of the present invention may comprise of genomic DNA sequence of about 1.6kb upstream from the start codon of SEQ ID NO: 8.

25 In additional embodiments, the present invention includes constructs comprising *TAA1* promoter sequences in operative association with an open reading frame, or a part thereof or a complement thereof, for use in modifying anther, tapetum or pollen metabolism. The constructs may be transformed into plants to generate transgenic plants with altered characteristics. For example, the invention
30 encompasses transgenic plants transformed with a construct having a *TAA1*

promoter or part thereof in operative association with an anther or pollen
inactivating gene, wherein expression of the open-reading frame induces male
sterility of the transgenic plant. Alternatively, the open-reading frame may
encode a transposase, and expression of the open-reading frame may induce an
5 increased rate of genomic DNA rearrangement in anther or pollen cells of the
transgenic plant. Alternatively, the open-reading frame may encode a peptide
suitable for use as a nutritional or pharmaceutical agent, the peptide being
expressed in anthers or pollen of the transgenic plant. Alternatively, the open-
reading frame may encode a peptide required for the production of a nutritional
10 or pharmaceutical agent, or a protein that inhibits the production and / or
accumulation of an unwanted substance selected from the group consisting of a
toxin, and an allergen, or a peptide for altering the cross-pollination specification
of the transgenic plant. Alternatively, the open reading frame may be oriented for
antisense expression within the construct, thereby inducing antisense repression
15 of endogenous gene expression within the anthers, tapetum or pollen of the
transgenic plant.

The present invention further provides, in alternative embodiments, for a means
for generating fatty alcohols that may be used as nutritional or pharmaceutical
20 agents. The fatty alcohols may be purified from extracts of the transgenic plants
using techniques that are well known in the art. Preferably, the fatty alcohols
generated by the transgenic plants of the present invention include Octacosanol; a
fatty alcohol known to produce health benefits including enhances physical
endurance and reproductive health. Moreover, in another preferred embodiment,
25 the transgenic plants of the present invention may be used to generate fatty
alcohols for the washing and cleaning industry. In alternative embodiments, the
transgenic plants of the present invention may bear fruit with increased levels of
fatty alcohols, wherein the fruit include wax derived from the fatty alcohols to
help preserve the fruit and improve the aesthetic appeal of the fruit, thereby
30 improving shelf life. The increased levels of wax production in the plants of the

present invention are further predicted to confer enhanced properties such as reduced rates of moisture loss, and increased resistance to pests.

5 The invention further encompasses the fatty alcohols derived or extracted from the transgenic plants or other transformed organisms (e.g. bacteria) of the present invention, and their use, for example as a wax, as a cleaning agent, as a cosmetic agent, as a dermatological agent, as a pharmaceutical agent, or as a nutritional agent.

10 The invention further encompasses pharmaceutical and nutritional compositions and agents comprising the plant extracts and fatty alcohols obtained from the transgenic plants of the present invention, as well as methods for treating or preventing a medical condition, or for providing a dietary supplement, by the administration of the plant extracts or fatty alcohols of the present invention.

15 The invention further encompasses method for the production and isolation of fatty alcohols, characterized in that the method comprises the steps of:
transforming an organism with a construct comprising a *TAA1* gene sequence, or part thereof, or complement thereof in accordance with the present invention;
20 growing or propagating said organism containing said construct; and
extracting said fatty alcohols from said organism. Preferably, the organism is an *E.coli* bacterium, such that recombinant *E.coli* comprising increased or altered levels of fatty alcohols may be cultured and harvested. In an alternative embodiment, the organism may comprise a plant or a plant embryo, preferably a
25 tobacco plant or tobacco plant embryo, that is induced to express the construct and generate increased or altered levels of fatty alcohols. Similarly, such transgenic plants may be grown and / or propagated thereby allowing plant extracts to be harvested and fatty alcohols to be purified by standard techniques.

The invention further encompasses a method of inducing dwarfism in a plant, characterized in that the method comprises the steps of:
transforming a plant cell, plant embryo or plant with a construct according to the present invention; and
5 growing or propagating said plant cell, plant embryo, or plant, thereby generating a plant expressing a DNA sequence encoded by said construct, said plant having a reduced size compared to an unmodified plant.

10 4. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 (a) schematically illustrates the genomic organization of *TAA1a*, *TAA1b*, and *TAA1c*. Triangles and rectangles represent introns and exons, respectively. The length (bp: base pair) of each intron and exon is shown above and in the
15 corresponding triangle and rectangle. The stippled and hatched triangles indicate a very long intron and an intron with alternative insertion position in *TAA1b*, respectively. The putative translation start (AUG) and stop (TGA) codons, and 5' and 3' UTR (untranslational region) are given. (A)_n represents a poly(A) tail. For clarity, cDNA sequences and introns are not drawn to scale.

20 Figure 1 (b) provides a genomic DNA blot analysis of different wheat species. Molecular size makers are indicated at left in kilobases. Sources of DNA are shown. Total DNA (10 µg) was digested with *Eco*RI (E), *Bam*HI (B) and *Hind*III (H), separated on a 1% agarose gel, blotted onto a nylon membrane, probed with
25 the coding region of *TAA1a*, and visualized by exposure to an x-film.

Figure 2 (a) demonstrates anther-specific expression of *TAA1*. R, roots; S, stems; L, leaves; A, anthers; O, ovary; G, glume and pilea. Northern blot analysis of *TAA1* expression in wheat. Total RNA (about 5 µg) purified from root, stem, leaf,
30 anther, ovary, and pilea and glume was loaded. A 0.7 kb fragment of the *TAA1a*

cDNA resulting from 5' RACE was used as a probe. The estimated size of hybridizing RNA species is shown to the left side. Underneath is the same blot hybridized with a 28S rRNA probe.

- 5 Figure 2 (b) demonstrates anther-specific expression of *TAA1*. R, roots; S, stems; L, leaves; A, anthers; O, ovary; G, glume and pilea. RT-PCR amplification of cDNA derived from different wheat tissues. Underneath is the same cDNA amplified with a pair of primers to a glyceraldehyde-3-phosphate dehydrogenase gene (GPD).

10

Figure 3 *In situ* RNA hybridization. The cross-sections of wheat flower buds were hybridized with *TAA1* anti-sense and sense transcript. Hybridization was shown by the formation of a dark bluish precipitate. Solid arrow head: tapetum; unfilled arrow head: micropore; m, microspores; ov, ovary; and ps, pollen sac.

15 Scale bars=100µm.

(a) probed with a *TAA1a* sense transcript.

(b) probed with a *TAA1* antisense transcript.

(c) sectioned at stage pre-meiosis, probed with a *TAA1* antisense transcript.

(d) sectioned at stage young microspore, probed with a *TAA1* antisense transcript.

- 20 (e) sectioned at stage vacuolated microspore, probed with a *TAA1* antisense transcripts.

Figure 4 Immunocytochemical detection of the TAA1 protein on the wheat anthers. The sections were immunoblotted with either pre-immune serum or TAA1a antiserum. Positive antibody recognition was shown by the formation of bluish deposits. M, microspores; ps, pollen sac, arrow heads, tapetum. Scale bars=100µm.

(a) immunoreacted with pre-immune serum (control).

(b) with TAA1a antiserum.

- 30 (c) an enlarged pollen sac of (a) (control).

(d) sectioned at the young microspore stage and immunoreacted with TAA1a antiserum.

(e) sectioned at the vacuolated microspore stage.

5 Figure 5 (a). Amino acid sequence comparison and phylogenetic analysis of TAA1. Pair-wise alignment of the amino acid sequence of TAA1a with that of FAR according to Pearson and Lipman (1998). Δ represents gaps which are introduced to allow the best matches. The dashes in FAR indicate the identical residues to TAA1a. Two potential transmembrane helixes predicted by Metz et
10 al. (2000) are underlined.

Figure 5 (b). Phylogenetic analysis of TAA1 and its related genes. The sequences of all related genes were obtained from public databases and refer to the following: FAR, the jojoba acyl coenzyme A reductase (accession no.
15 AF149917); MS2-like; a predicted gene from Arabidopsis (accession no. AB012244); MS2, the Arabidopsis male sterility 2 gene (accession no. S33804); B-MS2, the Brassica MS2 gene (accession no. T08096).

Figure 6 (a) Fatty alcohols in transgenic seeds and *E. coli*. Fatty alcohol content
20 in the tobacco seeds transformed with the *Napin-TAA1a* chimeric gene. The amounts of fatty alcohols obtained from GC analysis were normalized against the internal standard beta-sitosterol. The y-axis of the graph illustrates percentage 'FA' of the relative amounts of fatty alcohols to beta-sitosterol (%). Line 723-0-D was transformed with the control vector. All the remaining (477-0-4, 477-0-18,
25 477-0-2, and 477-0-10) were the *Napin-TAA1a* transgenic lines.

Figure 6 (b) Gas chromatography (GC) analysis of fatty alcohol amounts and compositions in bacterial cells without ((i) upper graph), or with ((ii) lower graph) expression of *TAA1a*. t = retention time in minutes, and CL = chain
30 length of fatty alcohol standards.

Figure 7. Over-expression of *TAA1* results in significant dwarfism in transgenic tobacco. Vector: transgenic plants containing NPTII resistant gene only; 35S::TAA1: transgenic containing both NPTII resistant gene and 35S::*TAA1a* chimeric gene. In this example, *TAA1* was over-expressed constitutively. (a) Three-week old seedlings in MS medium. (b) Plants three weeks after transplanting in a greenhouse. (c) Plants two months after transplanting in greenhouse.

Figure 8 Transient expression assay of *TAA1* promoter specificity. Hand cross-section of Daylily flower buds were bombarded with microprojectiles coated with either the CaMV35S-*uidA* (a) or *TAA1-uidA* chimeric genes (b). 35S-GUS transient expression was observed in anther walls, filaments, and petals (a). In contrast, TAA1-GUS transient expression limited in microspores and tapetum (arrow) (b). an: Anther, f: Filament, m: microspores, pe: petal, ps: pollen sac. Scale bars= 1 mm.

Figure 9 GUS expression pattern in transgenic tobacco anthers. (a) and (b) show GUS assays on hand cross-sections of anthers at different developmental stages of a transgenic plant containing a *TAA1-uidA* chimeric gene. (a) at the tetrad stage and (b) at the microspore separation stage. (c) and (d) show paraffin cross-sections of anthers of transgenic plants containing a 35S-*uidA* chimeric gene and a *TAA1-uidA* chimeric gene, respectively. aw: anther wall; cn: connective tissue; ep: epidermis; m: microspores; t: tapetum. Scale bars=200µm.

6. GLOSSARY OF TERMS

Amplification of DNA / amplified DNA: "amplified DNA" refers to the product of nucleic-acid amplification of a target nucleic-acid sequence. Nucleic-acid amplification can be accomplished by any of the various nucleic-acid

amplification methods known in the art, including the polymerase chain reaction (PCR). A variety of amplification methods are known in the art and are described, inter alia, in U.S. Pat. Nos. 4,683,195 and 4,683,202, and in Innis et al. (eds.), PCR Protocols: A Guide to Methods and Applications, Academic Press,
5 San Diego, 1990.

Construct: A construct comprises a vector and a DNA molecule operatively linked to the vector, such that the vector and operatively linked DNA molecule can be replicated and transformed as required.

10

Expression: The generation of a protein product derived from a DNA sequence encoding the protein, comprising a combination of transcription and translation.

Homologous: DNA or peptide sequences exhibiting similarity to another DNA or
15 peptide sequences in terms of the chemical nature, order and position of the individual residues relative to one another in the sequence. For the purposes of this application, unless stated otherwise homology is characterized according to BLAST search results, wherein a best-fit sequence alignment is obtained. In this way, sequences comprising residues that are similar or identical may be aligned,
20 and gaps provided as necessary. Homology is therefore expressed as a percentage of similarity or identity, wherein similarity encompasses both similar and identical residues. Unless stated otherwise, all BLAST searches were carried out using default parameters: e.g. gaps permitted, E-value =1, organism selected as required, filter for low complexity, standard genetic code, BLOSUM62 general
25 purpose matrix; for more information see
<http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/tut1.html>.

Identity: Comparison of homologous DNA or peptide sequences provides identification of residues that are identical in the same relative position of the
30 sequence, following best fit alignment. For the purposes of this application,

unless stated otherwise, homology, best fit alignment and identity are calculated according to BLAST search results (BLAST searching is available, for example, from the following website: <http://www.ncbi.nlm.nih.gov/BLAST/>). Identity is provided as a percentage, indicating the percentage of residues that are identical
5 along the sequences under comparison, excluding regions of gaps between the aligned sequences. BLAST searching permits a standard alignment configuration to automatically take into account regions of gaps or truncations between sequences, thereby providing a 'best fit' alignment.

10 Isolated: A nucleotide or peptide is "isolated" if it has been separated from other cellular components (nucleic acids, lipids, carbohydrates, and other nucleotides or peptides) that naturally accompany it. Such a nucleotide or peptide can also be referred to as "pure" or "homogeneous" or "substantially" pure or homogeneous. Thus, a nucleotide or peptide which is chemically synthesized or recombinant is
15 considered to be isolate. A nucleotide or peptide is isolated when at least 60-90% by weight of a sample is composed of the nucleotide or peptide, preferably 95% or more, and more preferably more than 99%. Protein purity or homogeneity is indicated, for example, by polyacrylamide gel electrophoresis of a protein sample, followed by visualization of a single peptide band upon staining the
20 polyacrylamide gel; high-performance liquid chromatography; or other conventional methods. The peptides of the present invention can be purified by any of the means known in the art. Various methods of protein purification are described, e.g., in Guide to Protein Purification, in Deutscher (ed.), Meth. Enzymol. 185, Academic Press, San Diego, 1990; and Scopes, Protein
25 Purification: Principles and Practice, Springer Verlag, New York, 1982.

Operably linked: two nucleotide sequences are operable linked if the linkage allows the two sequences to carry out their normal functions relative to each other. For instance, a promoter region would be operably linked to a coding
30 sequence if the promoter were capable of effecting transcription of that coding

sequence, and the coding sequence encoded a product intended to be expressed in response to the activity of the promoter.

Organ: A specific region of a plant defined in terms of structure and function, for example, a stem, a leaf, an anther, a pollen grain, or a root.

Promoter: A recognition site on a DNA sequence or group of DNA sequences that provides at least one expression control element for a gene encoding a polypeptide, and to which RNA polymerase specifically binds and initiates RNA synthesis (transcription) of the gene.

Stringent conditions, or stringent hybridization conditions: includes reference to conditions under which a probe will hybridize to its target sequence, to a detectably greater degree than other sequences (e.g. at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. Generally, stringent conditions are selected to be about 5 ° C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g. 10 to 50 nucleotides) and at least about 60°C for long probes (e.g. greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 2X SSC at 50°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60°C. Hybridization

procedures are well-known in the art and are described in Ausubel et al., (Ausubel F.M., et al., 1994, Current Protocols in Molecular Biology, John Wiley & Sons Inc.).

- 5 Transformation: Modification of a cell by the introduction of exogenous DNA sequence (e.g. a vector or recombinant DNA molecule).

Transgenic: A cell or organism derived from a process of cellular transformation, wherein the cell or organism comprises the introduced exogenous DNA molecule
10 not originally present in a non-transgenic cell or organism.

Transgenic plant: A plant or progeny thereof derived from a transformed plant cell or protoplast, wherein the plant DNA contains an introduced exogenous DNA molecule not originally present in a native, non-transgenic plant of the
15 same strain. The terms “transgenic plant” and “transformed plant” have sometimes been used in the art as synonymous terms to define a plant whose DNA contains an exogenous DNA molecule. However, it is thought more scientifically correct to refer to a regenerated plant or callus obtained from a transformed plant cell or protoplast as being a transgenic plant.

20

Vector: A DNA molecule capable of replication in a host cell and/or to which another DNA segment (or insert) can be operatively linked so as to bring about replication of the attached insert. A plasmid is an exemplary vector. Moreover, a vector may include promoter sequence to facilitate expression of an open reading
25 frame present in the DNA insert. All vectors used for the present application were generated by the inventors, with the exception of: T/A vectors (Invitrogen), pRSET A (Invitrogen), phagemids (Stratagene), pRD400 and pRD410 (Datla et al. 1992), pHS724 (Huang et al., 2000), pJOY43 (Nair et al., 2000).

7. DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

To support this application, two deposits of biological material have been made
5 under the Budapest Treaty regarding Deposits of Biological Material. The
deposits were made at the International Depository Authority of Canada, Bureau
of Microbiology, Health Canada, Winnipeg, Manitoba, Canada, on June 7, 2001,
under accession numbers IDAC 070601-2 and IDAC 070601-1. The deposits
both comprise *E.coli* bacterial cells, strain DH5 α , transformed with constructs
10 comprising DNA sequence of the present invention. Deposit number IDAC
070601-2 consists of DH5 α cells transformed with pAMW133 comprising the
full length coding region of *TAA1a* cDNA. Deposit number IDAC 070601-1
consists of DH5 α cells transformed with pAMW170 comprising the promoter
region of the *TAA1b* gene.

15 *Nucleotide sequences encompassed by the present invention*

The present invention provides a polynucleotide molecule comprising nucleotide
20 sequences derived from the *TAA1* family. The genetic sequences encompassed
by the present invention include, but are not limited to, *TAA1a* cDNA (SEQ ID
NO: 1), *TAA1b* cDNA (SEQ ID NO: 3), *TAA1c* cDNA (SEQ ID NO: 5), *TAA1a*
genomic DNA (SEQ ID NO: 7), *TAA1b* genomic DNA (SEQ ID NO: 8), and
TAA1c genomic DNA (SEQ ID NO: 9).

25 Whilst the present invention discloses polynucleotide sequences for three closely
related genes, homologous nucleotide sequences encoding peptides with
significant amino acid sequence identity to those encoded by SEQ ID NOS: 1, 3,
5, 7, 8, and 9, can be readily obtained in accordance with the teachings of the
30 present application (and references disclosed herein), and are encompassed within
the scope of the present invention. In this regard, nucleotide sequences of the
present invention can be used to produce (degenerate) nucleotide probes, for the

purposes of screening cDNA and genomic DNA libraries of various plant species. Related techniques are well understood in the art, for example as provided in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y.(1989). In this way, sequences homologous to those of the present application are readily obtainable. For this reason, it is the intention of the present invention to encompass polynucleotide molecules comprising DNA sequences that encode peptides with significant sequence identity to those disclosed in the present application, wherein SEQ ID NOS: 1, 3, 5, 7, 8, or 9, or parts thereof, may be utilized as polynucleotide probes to search for and isolate homologous polynucleotide molecules. Moreover, polynucleotides encoding proteins with significant sequence identity to those of the present application are expected give rise to similar protein products with similar biochemical characteristics, to those described in the present application. Indeed, such techniques were used by the inventors to isolate the various *TAA1* cDNA and genomic DNA homologous sequences disclosed herein. More details in this regard are provided in the examples.

The present invention therefore encompasses DNA sequences obtained by techniques known in the art for isolating homologous DNA sequences, wherein the techniques utilize degenerate oligonucleotide probes derived from a sequence selected from SEQ ID NO:1, 3, 5, 7, 8, and 9, or parts thereof. The degree of amino acid sequence identity will vary for each identified sequence. It is the intention of the present invention to encompass polynucleotide sequences comprising at least 50% sequence identity with regard to the peptide sequences encoded by the corresponding polynucleotides. Without wishing to be bound by theory, it is generally expected in the art that enzymes with at least 50% identity can have enzymatic activities that are similar in scope. In this regard, the essential structural features of the enzyme are preserved to scaffold the conformation of the catalytic site of the enzyme. Therefore, the present invention encompasses polynucleotide molecules derived by screening genomic and cDNA

libraries of plant types including wheat and other species, using degenerate DNA probes derived from the sequences disclosed in the present application. Such species include, but are not restricted to: rye, barley, rice and other grasses, and monocots such as maize, and lily.

5

The present invention also encompasses polynucleotide sequences obtained by screening DNA libraries using degenerate oligonucleotide probes derived from the polynucleotides of the present invention, wherein the sequences encode peptides comprising at least 70% amino acid sequence identity to peptides encoded by SEQ ID NOS: 1, 3, 5, 7, 8, and 9. In this regard, homologous proteins with at least 70% predicted amino acid sequence identity are expected to encompass proteins with similar fatty acyl Co-A reductase activity as those defined by the present invention, but possibly with altered substrate specificity. Such proteins may be derived from related species of plant.

15

The present invention also encompasses polynucleotide sequences encoding peptides comprising at least 90%, 95% or 99% sequence identity to the peptides encoded by SEQ ID NOS: 1, 3, 5, 7, 8, and 9. This class of related proteins is intended to include close gene family members with very similar or identical catalytic activity. In addition, peptides with 90%, 95% or 99% amino acid sequence identity may be derived from functional homologues of similar species of plant, or from directed mutations to the sequences disclosed in the present application.

20

25 *Isolation of TAA1 cDNA and genomic DNA homologues*

With the provision of several *TAA1* cDNA and genomic DNAs, the polymerase chain reaction (PCR) may now be utilized in a preferred method for isolating further *TAA1* homologous nucleotide sequences from wheat and other species of plant. PCR amplification of the *TAA1* cDNA sequence may be accomplished either by direct PCR from a plant cDNA library or by

30

Reverse-Transcription PCR (RT-PCR) using RNA extracted from plant cells as a template. Methods and conditions for both direct PCR and RT-PCR are known in the art and are described in numerous standard textbooks. Similarly, the TAA1 genomic sequences may be amplified directly from genomic DNA
5 extracted from plants, or from plant genomic DNA libraries. Amplification may be used to obtain the full length cDNA or genomic sequence, or may be used to amplify selected portions of these molecules (for example for use in antisense constructs).

10 Moreover, the well known technique of chromosome walking can be readily used to isolate regions of genomic DNA that are 5' or 3' to the coding region of the gene. The technique of chromosome walking is described, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y.(1989). The present disclosure includes analysis
15 of a region upstream of to the *TAA1b* genomic DNA start codon, that is suitable for use as an anther specific promoter.

The selection of PCR primers will be made according to the portions of the *TAA1* nucleic acids which are to be amplified, including full-length *TAA1* clones.
20 Variations in amplification conditions may be required to accommodate primers of differing lengths; such considerations are well known in the art.

Oligonucleotides which are derived from the *TAA1* nucleic acid sequences described herein, and which are suitable for use as PCR primers to amplify
25 additional *TAA1* nucleic acid sequences are encompassed within the scope of the present invention. Preferably, such oligonucleotide primers will comprise a sequence of 15-20 consecutive nucleotides of the *TAA1* nucleic acid sequences. To enhance amplification specificity, primers comprising at least 20-30 consecutive nucleotides of these sequences may also be used.

30

With the provision herein of the *TAA1* nucleic acid sequences, the cloning by standard methodologies of corresponding cDNAs and genes from other ecotypes and plant species, as well as polymorphic forms of the disclosed sequences is now enabled. Thus, the present invention includes methods of isolating a
5 nucleotide sequence encoding a TAA1 enzyme from a plant. Both conventional hybridization and PCR amplification procedures may be utilized to clone such sequences. Common to both of these techniques is the hybridization of probes or primers derived from the disclosed *TAA1* nucleic acid sequences to a target nucleotide preparation, which may be, in the case of conventional hybridization
10 approaches, a cDNA or genomic library or, in the in the case of PCR amplification, extracted genomic DNA, mRNA, a cDNA library or a genomic library.

Direct PCR amplification may be performed on cDNA libraries prepared from
15 the plant species in question, or RT-PCR may be performed using mRNA extracted from the plant cells using standard methods. PCR primers will comprise at least 15 consecutive nucleotides of the *TAA1* nucleic acid sequences. One of skill in the art will appreciate that sequence differences between the disclosed *TAA1* nucleic acid sequences and the target gene to be amplified may result in
20 lower amplification efficiencies. To compensate for this, longer PCR primers or lower annealing temperatures may be used during the amplification cycle. Where lower annealing temperatures are used, sequential rounds of amplification using nested primer pairs may be necessary to enhance specificity.

25 *Generation of TAA1 variants and mutants*

For conventional hybridization techniques, the hybridization probe is preferably labeled with a detectable label such as a radioactive label, and the probe is of at least 20 nucleotides in length. As is well known in the art, increasing length of
30 hybridization probes tends to give enhanced specificity. The labeled probe

derived from, for example, the TAA1 cDNA sequence may be hybridized to a plant cDNA or genomic library and the hybridization signal detected using means known in the art. The hybridizing colony or plaque (depending on the type of library used) is then purified and the cloned sequence contained in that colony or
5 plaque isolated and characterized.

It will also be understood to a person of skill in the art that site-directed mutagenesis techniques are readily applicable to the polynucleotide sequences of the present invention. Related techniques are well understood in the art, for
10 example as provided in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y.(1989). In this regard, the present invention teaches the isolation and characterization of the DNA sequences as provided as SEQ ID NOS: 1, 3, 5, 7, 8, and 9. However, the present invention is not intended to be limited to these specific sequences.
15 Numerous directed mutagenesis techniques would permit the non-informed technician to alter one or more residues in the nucleotide, thus changing the subsequently expressed polypeptide sequences. Moreover, commercial 'kits' are available from numerous companies that permit directed mutagenesis to be carried out (available for example from Promega and Biorad). These include the
20 use of plasmids with altered antibiotic resistance, uracil incorporation and PCR techniques to generate the desired mutations. The mutations generated may include point mutations, deletions and truncations as required. The present invention is therefore intended to encompass corresponding mutants of the *TAA1* cDNA and genomic DNA sequences disclosed in the present application.

25 The mutated variants of the sequences of the present application are predicted to include enzymes with reduced or increased fatty acyl Co-A reductase activity, as well as altered substrate specificity. Such mutants may confer advantageous properties to subsequently transformed transgenic cell lines and plants. For
30 example, a transgenic plant comprising a construct overexpressing an inactive

mutant of the enzymes of the present invention can be expected to have a significantly altered profile of lipid constituents, including a possible reduction in fatty alcohol content. In contrast, the expression of mutant fatty acyl Co-A reductase enzymes with increased catalytic turnover are expected to give rise to transgenic plants with an high level of fatty alcohols. Mutant fatty acyl Co-A reductase enzymes with altered substrate specificity will likely be useful in altering the relative quantities of lipid metabolism products generated in a correspondingly transformed plant, or altering the distribution of the lipid metabolism products within the organs of the plant.

10

Generation of constructs comprising TAA1 sequence

The polynucleotide sequences of the present invention can be ligated into suitable vectors before transfer of the genetic material into plants. For this purpose, standard ligation techniques that are well known in the art may be used. Such techniques are readily obtainable from any standard textbook relating to protocols in molecular biology, and suitable ligase enzymes are readily available from commercial sources. A number of recombinant vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described, which are also readily available from commercial sources. Typically, plant transformation vectors include one or more cloned plant genes (or cDNAs) under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant transformation vectors typically also contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

15

20

25

As noted above, the particular arrangement of the *TAA1* nucleic acid in the transformation vector will be selected according to the expression of the nucleic acid desired.

- 5 Where enhanced fatty alcohol synthesis is desired, the *TAA1* nucleic acid may be operably linked to a constitutive high-level promoter such as the CaMV 35S promoter. Modification of fatty alcohols synthesis may also be achieved by introducing into a plant a transformation vector containing a variant form of the *TAA1* nucleic acid, for example a form which varies from the exact nucleotide
10 sequence of the *TAA1* nucleic acid, but which encodes a protein that retains the functional characteristic of the TAA1 protein, i.e., fatty acyl Co-A reductase activity.

- In contrast, a reduction of fatty alcohol synthesis may be obtained by introducing
15 antisense constructs based on the *TAA1* nucleic acid sequence into plants. For antisense suppression, the *TAA1* nucleic acid is arranged in reverse orientation relative to the promoter sequence in the transformation vector. The introduced sequence need not be the full length *TAA1* nucleic acid, and need not be exactly homologous to the *TAA1* nucleic acid. Generally, however, where the introduced
20 sequence is of shorter length, a higher degree of homology to the native *TAA1* sequence will be needed for effective antisense suppression. Preferably, the introduced antisense sequence in the vector will be at least 30 nucleotides in length, and improved antisense suppression will typically be observed as the length of the antisense sequence increases. Preferably, the length of the antisense
25 sequence in the vector will be greater than 100 nucleotides. Transcription of an antisense construct as described results in the production of RNA molecules that are the reverse complement of mRNA molecules transcribed from the endogenous *TAA1* gene in the plant cell. Although the exact mechanism by which antisense RNA molecules interfere with gene expression has not been elucidated,
30 it is believed that antisense RNA molecules bind to the endogenous mRNA

molecules and thereby inhibit translation of the endogenous mRNA or trigger the degradation of mRNA, or inhibit transcription by causing methylation of the gene. A variation of the antisense suppression includes RNAi strategy as published in the literature under various names such as double stranded (dsRNA) RNA suppression.

Suppression of endogenous *TAA1* gene expression can also be achieved using ribozymes. Ribozymes are synthetic RNA molecules that possess highly specific endoribonuclease activity. The production and use of ribozymes are disclosed in U.S. Pat. Nos. 4,987,071 to Cech and 5,543,508 to Haselhoff, which are hereby incorporated by reference. The inclusion of ribozyme sequences within antisense RNAs may be used to confer RNA cleaving activity on the antisense RNA, such that endogenous mRNA molecules that bind to the antisense RNA are cleaved, which in turn leads to an enhanced antisense inhibition of endogenous gene expression.

Constructs in which the *TAA1* nucleic acids (or variants thereon) are over-expressed may also be used to obtain co-suppression of the endogenous *TAA1* gene in the manner described in U.S. Pat. No. 5,231,021 to Jorgensen. Such co-suppression (also termed sense suppression) does not require that the entire *TAA1* nucleic acid be introduced into the plant cells, nor does it require that the introduced sequence be exactly identical to the *TAA1* nucleic acid. However, as with antisense suppression, the suppressive efficiency will be enhanced as (1) the introduced sequence is lengthened and (2) the sequence similarity between the introduced sequence and the endogenous *TAA1* gene is increased.

Transformation of TAA1 constructs

The present invention also encompasses a plant cell transformed with a nucleotide sequence of the present invention, and as well as plants derived from

propagation of the transformed plant cells. Numerous methods for plant transformation have been developed, including biological and physical, plant transformation protocols. See, for example, Miki et al., "Procedures for Introducing Foreign DNA into Plants" in *Methods in Plant Molecular Biology and Biotechnology*, Glick, B. R. and Thompson, J. E. Eds. (CRC Press, Inc., Boca Raton, 1993) pages 67-88. In addition, expression vectors and in vitro culture methods for plant cell or tissue transformation and regeneration of plants are available. See, for example, Gruber et al., "Vectors for Plant Transformation" in *Methods in Plant Molecular Biology and Biotechnology*, Glick, B. R. and Thompson, J. E. Eds. (CRC Press, Inc., Boca Raton, 1993) pages 89-119.

The following are examples, and are not limiting:

A. *Agrobacterium*-mediated Transformation: One method for introducing an expression vector into plants is based on the natural transformation system of *Agrobacterium*. See, for example, Horsch et al., *Science* 227: 1229 (1985). *A. tumefaciens* and *A. rhizogenes* are plant pathogenic soil bacteria which genetically transform plant cells. The Ti and Ri plasmids of *A. tumefaciens* and *A. rhizogenes*, respectively, carry genes responsible for genetic transformation of the plant. See, for example, Kado, C. I., *Crit. Rev. Plant. Sci.* 10: 1 (1991). Descriptions of *Agrobacterium* vector systems and methods for *Agrobacterium*-mediated gene transfer are provided by Gruber et al., *supra*, Miki et al., *supra*, and Moloney et al., *Plant Cell Reports* 8: 238 (1989). Bechtold et al., *C. R. Acad. Sci. Paris Life Sciences*, 316:1194-9 (1993).

B. Direct Gene Transfer: Several methods of plant transformation, collectively referred to as direct gene transfer, have been developed as an alternative to *Agrobacterium*-mediated transformation. A generally applicable method of plant transformation is microprojectile-mediated transformation wherein DNA is carried on the surface of microprojectiles measuring 1 to 4 μm . The expression

- vector is introduced into plant tissues with a biolistic device that accelerates the microprojectiles to speeds of 300 to 600 m/s which is sufficient to penetrate plant cell walls and membranes. Sanford et al., *Plant Sci. Technol.* 5: 27 (1987), Sanford, J. C., *Trends Biotech.* 6: 299 (1988), Klein et al., *Bio/Technology* 6: 559-563 (1988), Sanford, J. C., *Physiol Plant* 79: 206 (1990), Klein et al., *Biotechnology* 10: 268 (1992). See also U.S. Pat. No. 5,015,580 (Christou, et al), issued May 14, 1991; U.S. Pat. No. 5,322,783 (Tomes, et al.), issued Jun. 21, 1994.
- 10 C. Other methods: Another method for physical delivery of DNA to plants is sonication of target cells. Zhang et al., *Bio/Technology* 9: 996 (1991). Alternatively, liposome or spheroplast fusion have been used to introduce expression vectors into plants. Deshayes et al., *EMBO J.*, 4: 2731 (1985), Christou et al., *Proc Natl. Acad. Sci. U.S.A.* 84: 3962 (1987). Direct uptake of
- 15 DNA into protoplasts using CaCl_2 precipitation, polyvinyl alcohol or poly-L-ornithine have also been reported. Hain et al., *Mol. Gen. Genet.* 199: 161 (1985) and Draper et al., *Plant Cell Physiol.* 23: 451 (1982). Electroporation of protoplasts and whole cells and tissues have also been described. Donn et al., In *Abstracts of VIIth International Congress on Plant Cell and Tissue Culture*
- 20 IAPTC, A2-38, p 53 (1990); D'Halluin et al., *Plant Cell* 4: 1495-1505 (1992) and Spencer et al., *Plant Mol. Biol.* 24: 51-61 (1994).

Following transformation of target cell(s) or tissues, expression of the above-described selectable marker genes allows for preferential selection of transformed

25 cells, tissues and/or plants, using regeneration and selection methods now well known in the art.

The foregoing methods for transformation would typically be used for producing a transgenic variety. The transgenic variety could then be crossed, with another

(non-transformed or transformed) variety, in order to produce a new transgenic variety.

Alternatively, a genetic trait which has been engineered into a particular line
5 using the foregoing transformation techniques could be moved into another line using traditional backcrossing techniques that are well known in the plant breeding arts. For example, a backcrossing approach could be used to move an engineered trait from a public, non-elite variety into an elite variety, or from a variety containing a foreign gene in its genome into a variety or varieties which
10 do not contain that gene. As used herein, "crossing" can refer to a simple X by Y cross, or the process of backcrossing, depending on the context. Once a transgenic plant has been established, it is important to determine the phenotype of the seeds of the plant.

15 Accordingly, in a preferred embodiment of the invention a method is provided for modifying the seed of a plant comprising the steps of:

(a) introducing into a plant cell capable of being transformed and regenerated into a whole plant a construct comprising, in addition to the DNA sequences required for transformation and selection in plants, a nucleotide
20 sequence in accordance with the nucleotide sequences encompassed by the present invention, operably linked to a promoter; and

(b) recovery of a plant which contains the nucleotide sequence.

The present invention therefore encompasses the transformation of a variety of
25 plant species, including woody, non-woody, fruit bearing and grass species, with the DNA sequences disclosed. Particularly preferred varieties include crucifer crops, tobacco, wheat, corn, sugar cane, and apple. The present invention is particularly considered to be useful in the generation of modified fruits such as apples, since increased expression of fatty acyl Co-A reductase enzymes of the
30 present invention is expected to increase the fatty alcohol concentration in the

fruits, thus providing the fruits with a more waxy texture, and a more aesthetically pleasing coating.

Fatty acid analysis and purification

5

Once a transgenic plant has been established, it is important to determine the fatty alcohol content of the plant, or various plant organs. For this purpose, several techniques are known in the art to for the analysis of the chemical content of plant material, and in particular, the lipid and fatty alcohol content of the plant.

10

These techniques include Gas Chromatography (GC), high performance liquid chromatography, and MS-GC, as well as other techniques that are familiar to those of skill in the art. Moreover, the fatty alcohol products may be extracted from the plant by any one of a range of techniques that are well known in the art for the purposes of lipid extraction.

15

One example of fatty alcohol purification from plant materials is outlined in the Experimental Procedures where fatty alcohols were purified for Gas Chromatography analysis. An alternative method was published by Miwa, T.K. 1971. Journal of The American Oil Chemists' Society. 48:259-264, in relation to jojoba oil analysis.

20

Production of recombinant TAA1 protein using heterologous expression systems

Many different expression systems are available for expressing cloned nucleic acid molecules. Examples of prokaryotic and eukaryotic expression systems that are routinely used in laboratories are described in Chapters 16-17 of Sambrook et al. (1989), which are herein incorporated by reference. Such systems may be used to express a TAA1 protein or derivatives thereof at high levels to facilitate purification and functional analysis of the enzyme. Apart from permitting the activity of the enzyme to be determined (which is particularly useful to assess the

25

30

activity of homologous and derivative proteins), heterologous expression facilitates other uses of the purified enzyme. For example the purified enzyme produced by recombinant means may be used to synthesize fatty alcohols and other fatty acid metabolites in vitro, particularly radio- or fluorescent-labeled forms of fatty alcohols and metabolites. These molecules may be used as tracers to determine the location in plant tissues and cells of fatty alcohols and their metabolites. The purified recombinant enzyme may also be used as an immunogen to raise enzyme-specific antibodies. Such antibodies are useful as both research reagents (such as in the study of fatty alcohol regulation in plants) as well as diagnostically to determine expression levels of the enzyme in agricultural products, including pollen.

By way of example only, high level expression of the TAA1 protein may be achieved by cloning and expressing the cDNA in yeast cells using the pYES2 yeast expression vector (Invitrogen, San Diego, Calif.). Secretion of the recombinant TAA1 from the yeast cells may be achieved by placing a yeast signal sequence adjacent to the *TAA1* coding region. A number of yeast signal sequences have been characterized, including the signal sequence for yeast invertase. This sequence has been successfully used to direct the secretion of heterologous proteins from yeast cells. Alternatively, the enzyme may be expressed at high level in standard prokaryotic expression systems, such as *E. coli*.

Homology of TAA1 nucleic acids to other plant genes

It is predicted that the *TAA1* genes are the first anther-specific genes in wheat to be reported. TAA1 appears to be related to the jojoba FAR (reported by Metz et al., 2000). Both have an M_r of $\sim 58,000$, and share $\sim 44\%$ aa identity and $\sim 63\%$ similarity. This FAR is the only biochemically characterized enzyme for which a deduced structure is available. It belongs to the category of alcohol-forming

FARs that produces fatty alcohols directly from fatty acyl CoA, one of the two penultimate substrates in wax biosynthesis (Kolattukudy, 1970). The other category of fatty acyl CoA reductases is smaller and produces aldehydes ($M_r \sim 30$ kDa) (Vioque and Kolattukudy, 1997), and thus TAA1 is further distinct from these. In jojoba, FAR was isolated from developing seeds. It is not clear if it is also expressed in the anther and other tissues.

The predicted *TAA1* gene products share a lesser, yet significant homology with two known anther-specific genes, the *Arabidopsis MS2* gene (Aarts et al., 1997) and the *Brassica MS2* gene (Aarts et al., 1997; Hodge et al., 1992). Both of them also share significant homology with FAR (~40% identity and ~59% similarity). Any functional implication of TAA1 and MS2 relationship must be considered in light of the observation that TAA1 shows a greater relationship to the jojoba FAR. The *Arabidopsis MS2* gene is required for pollen development (Aarts et al., 1993; Aarts et al., 1997). The post-meiotic, tapetal expression of *MS2* in *Arabidopsis* and that of *TAA1* in wheat are almost identical. Thus, despite the deviation in the deduced primary structures, both appear to be functionally similar. While a partial redundancy of *MS2* function has not been ruled out (Aarts et al., 1993; Aarts et al., 1995), the *Arabidopsis* genome database (*Arabidopsis thaliana* genome CD) was searched using the *MS2* aa sequence. Excluding the *MS2* itself, seven hypothetical proteins including two named male sterility 2 - like, four called acyl coA reductase-like, and one putative protein were found. These proteins are 37-42% identical to and 59-65% similar with the *MS2* aa sequence. These proteins range from 409 to 527 aa in length and also share aa homology to TAA1 at similar levels. Moreover, BLASTN searches using the *MS2* cDNA did not retrieve any more sequences than itself, indicating that at the nt level, the *MS2* does not share significant homology with these predictive genes. In contrast, these genes seem to be more closely related to each other as they conserve significant similarities both at the aa level and the nt level (data not shown). However, it is not know if these genes function in pollen development.

Aarts et al. (1993) identified a short segment of homology between a wheat mitochondrial sequence (Spencer et al., 1992) and the Arabidopsis MS2 at the deduced aa sequence level. This (93/153 aa) occurred over two stretches of the
5 mitochondrial sequence with an unrelated sequence flanked by hallmarks of nuclear splice junctions that connected the two parts. Since splicing in mitochondrial transcripts follows a different scheme, Aarts et al. (1997) proposed that the mitochondrial sequence is of nuclear origin that had recently migrated to the organellar genome. Were this the case, there should be a greater homology
10 between the nt sequence of *TAA1*, a wheat genomic sequence, and the wheat mitochondrial sequence. Instead, there was no significant homology even though the deduced TAA1a protein did show a similar rate of homology to a discontinuously translated polypeptide of the mitochondrial sequence at the same region as MS2 (93/153 aa). Therefore, the corresponding mitochondrial sequence
15 is more likely due to the biochemical convergence in evolution than a genealogical relationship.

This application provides convincing evidence that TAA1 is an FAR. FAR converts fatty acyl coA to fatty alcohol (Kolattukudy, 1970; Kolattukudy and
20 Rogers, 1986; Lardizabal et al., 2000; Metz et al., 2000). This is the first plant tapetum-specific gene which is enzymatically identified to be associated with lipid and wax biosynthesis. Pollen grains are coated with two layers of lipidic structures, i.e., the pollen outer wall (exine) and the pollen outmost coating (tryphine) that is overlaid on exine. Sporopollenin is the major constituent of
25 exine and contains metabolites derived from long chain fatty acids and phenylpropanoids (reviewed by Scott, 1994). Although long chain fatty lipids seem to be definitely required for the synthesis of sporopollenin, how sporopollenin is polymerized and what precursors participate in the polymerization still remain unclear. In crucifer plants such as *Arabidopsis* and
30 *Brassica*, the deposition of the exine takes place from the completion of meiosis

II, through the tetrad and ring-vacuolate stages, to the time of the first pollen mitosis (Piffanelli et al., 1998). During this process, the tapetum is performing very active lipid biosynthesis (Piffanelli et al., 1998). So, it is logically assumed that the tapetum plays a major role in exine formation. Prior to this report, the only functionally characterized anther tapetum-specific gene was the Arabidopsis *MS2* gene, whose expression pattern is concomitant with the formation of the pollen outer cell wall. Disruption of the *MS2* gene with a transposon results in male sterility. Pollen development in the *ms2* mutant shows most dramatic defect upon release from tetrads (Aarts et al., 1997). These pollens lack exine. Though the enzymatic nature of its encoded polypeptide has yet to be identified, it shares 40% aa sequence identity with FAR. The present application includes evidence that TAA1 has the *MS2* expression pattern during formation of microspore exine and its encoded polypeptide has fatty alcohol forming capacity. Thus, as proposed by Scott (1994) and Aarts et al. (1997), fatty alcohols, the TAA1 or *MS2* enzymatic products are likely to be the precursors for sporopollenin polymerization.

The outmost layer of the pollen grain is the pollen coating or tryphine derived from two tapetum-specific lipid-rich organelles, elaioplasts and tapetosomes (Hernández-Pinzón et al., 1999; Piffanelli et al., 1998; Ting et al., 1998; Wu et al., 1997). The former is a plastid with triacylglycerol (TAG) and neutral esters, and the later is a lipid body containing neutral lipids including TAG and wax esters, and also oleosin-like proteins. The main functions of tryphine include pollen-stigma recognition and subsequent pollen hydration (Piffanelli et al., 1998). Analysis of tryphine lipid fractions shows that the tryphine lipids contain TAG, triterpene esters, sterol esters and very long-chain wax esters (Bianchi et al., 1990; Preuss et al., 1993). Of these compounds, long-chain lipids and linear waxes are thought to be essential for the functions of the pollen coating (Lemieux, 1996; Mariani and Wolters-Arts, 2000; Negruk et al., 1996; Preuss et al., 1993). This is in agreement with the previous observations that wax defective

mutants such as the *cer1*, *cer2*, *cer3*, *cer6*, *cer8* and *cer10* mutants in *Arabidopsis* exhibit conditional male sterility (Hannoufa et al., 1993; Koornneef et al., 1989). Of these mutants, some have tryphine with smaller lipid droplets than wild-type pollen and some have tryphine without lipids. Recently, a complementation
5 experiment of the *cer6* mutants by transgenically expressing the *CER6* gene has shown that the two phenotypic effects, i.e. wax defection and male sterility cannot be rescued equally (Fiebig et al., 2000). *CER6* is identical to *CUT1* encoding an enzyme responsible for elongation of fatty acyl CoA, and silencing *CUT1* induces waxless stem and male sterility (Millar et al., 1999). Interestingly,
10 some fertility-restored lines (*CER6* transformants) of the *cer6* mutants still show wax-defective stem. Analysis on very long fatty lipids reveals that low amounts of long fatty lipids are sufficient for pollen hydration and germination, suggesting that this remarkable difference results from the different requirements for *CER6* activity on stems and the pollen coating (Fiebig et al., 2000). Apart from the
15 ingenious very long chain lipids, a class of exogenous TAGs which is absent in the pollen coating of *Arabidopsis* also can rescue the fertility of an *Arabidopsis* wax-defective mutant (Wolters-Arts et al., 1998). These findings raise a possibility that the tryphine function is dependent on the nature of the mixture of lipids including TAG, very long fatty acids and waxes in the tryphine. The
20 composition of and the relative amounts of each species of the lipid pool rather than a single macromolecule determine a functional pollen coating.

Apart from their presence in pollen lipidic structures (Bianchi et al., 1990), linear wax esters are also ubiquitously present in the plant cuticle (Piffanelli et al.,
25 1998; Post-Beittenmiller, 1996), indicating plant encoded FAR genes have evolved divergent expression mechanisms. Indeed, an alcohol-forming FAR was previously purified from pea leaves (Vioque and Kolattukudy, 1997). Since the lipid composition of tryphine is significantly different from that of the cuticle and even that of the intracellular contents of the pollen grains in the same plants
30 (Bianchi et al., 1989; Bianchi et al., 1990; Piffanelli et al., 1997), there must be a

mechanism by which the wax biosynthesis is spatially and temporally regulated. Searches of the Arabidopsis genome database identify 7 FAR-like hypothetical proteins excluding the MS2. It would be possible to investigate if these FAR-like genes contribute to divergent lipid biosynthesis in plants. Alternatively, isolation
5 and characterization of more FAR genes will definitely assist in understanding this complicate regulation mechanism.

The present application provides convincing evidence that TAA1 can reduce long chain acyl CoA to fatty alcohols. Fatty alcohols can be further esterified with
10 fatty acids to generate linear wax esters. Transgenic plants that overexpress TAA1 proteins via their natural (tapetum specific) promoters are predicted to have an increased consumption of fatty acyl CoA by TAA1 in the tapetum, which in turn may impact upon lipid-related biosynthesis in the anthers. In this regard, alteration of the lipid composition in the tapetum by TAA1 may be also required
15 for the tryphine development to assure its recognition and hydration function. Corresponding effects upon lipid metabolism are predicted to occur if the TAA1 protein is overexpressed in a plant organ other than the tapetum. For this reason, the present invention encompasses DNA constructs, and the corresponding transgenic plants transformed with the constructs, wherein the over- or under-
20 expression of TAA1-like proteins gives rise to altered lipid metabolism by virtue of an abnormal level of fatty acyl Co-A. Such modifications to lipid metabolism can have profound effects upon phenotype, developmental, reproductive, growth and structural characteristics of the plant. Moreover, the nature and impact of these effects are expected to depend upon the extent of TAA1 expression, and the
25 localisation of TAA1 expression to specific plant organs. Both of these factors are regulated in part the strength and specificity of the promoter.

Specific embodiments of the present invention are illustrated by way of the following examples:

Example 1 - The *TAA1* group of genes in wheat – identification and structural characteristics of the cDNA clones

RT-PCR experiments were conducted using an anther-specific cDNA library with
5 primers specific for the rice *PSI* gene (Zou et al., 1994). At a moderate annealing
temperature of 43°C a 0.7-kb amplicon was obtained from mRNA isolated from
anther but not from root, stem, leaf, glume and pilea tissues. DNA sequencing of
the amplicon *en masse* gave an unambiguous result indicating that the PCR
product was composed of a homogeneous sequence within the detection limits of
10 sequencing reaction. Since this amplicon was specific to anther mRNA, a full-
length cDNA clone encompassing the amplicon sequence was obtained by 5'-
and 3'- RACE. On probing an anther cDNA library with the full-length cDNA,
12 clones were identified and these were grouped into three groups according to
their restriction pattern (data not shown). The longest clones from each group
15 were studied further. 5'-RACE experiments did not produce further extensions,
suggesting that the longest clones were full-length cDNA clones and hereafter
referred as *TAA1a*, *TAA1b* and *TAA1c* (SEQ ID NOS: 1, 3, and 5 respectively) .
Both *TAA1a* cDNA (GenBank accession number AJ459249) and *TAA1c* cDNA
(GenBank accession number AJ459253) clones have a predicted open reading
20 frame (ORF) of 1524 nucleotides (nt) encoding 507 amino acids (aa) but with
different lengths of 5' UTRs (*TAA1a*: 69 nt and *TAA1c*: 93 nt) (Figure 1a). The
TAA1b cDNA (GenBank accession number AJ459251) ORF has a slightly larger
ORF (1569 nt) encoding 522 amino acids and a 5' UTR of 73 nt. The putative
polyadenylation signals, AATAA or TATAA, were found in the 3' UTR of all
25 three *TAA1* cDNAs. Database analysis on the deduced aa sequences encoded by
these three genes revealed that they all shared similarity to the fatty acyl-
coenzymeA reductase (FAR) gene of jojoba (Metz et al., 2000) and the *A.*
thaliana *MS2* gene (Aarts et al., 1993) (see later re.: Figure 5). On further
examination, the primers initially used contained at their 3' end a high level of

identity to two portions of the cDNA clone encompassing the 0.7 kb cDNA fragment.

Example 2 - *The TAA1 group of genes in wheat – identification and structural characteristics of the genomic DNA clones*

The genomic counterparts of the entire coding region of all three *TAA1* cDNAs were obtained by PCR amplification of the genomic DNA with primers based on the cDNAs. The genomic DNA sequences for *TAA1a*, *TAA1b*, and *TAA1c* are shown in SEQ ID NOS. 7, 8, and 9 respectively. The *TAA1a* genomic DNA has been assigned Genbank accession number AJ459250, the *TAA1b* genomic DNA has been assigned GenBank accession number AJ459252, and the *TAA1c* genomic DNA has been assigned GenBank Accession number AJ459254 (genomic sequences submitted to GenBank include only the genomic DNA regions encompassing coding sequence).

Nucleotide sequence analysis of the amplicons showed 7 introns interrupting the coding regions in all three genes. The length and composition varied among the three genes. The most significant difference was in the length of the second intron (1758 nt in *TAA1b*, but only 113 in *TAA1a* and 112 in *TAA1c*). The position of the 4th intron in *TAA1b* also deviated substantially (Figure 1 a).

Example 3 - *TAA1 genes are likely to exist as single copy per haploid genome*

Southern blot analysis of the wheats of different genetic constitution -- namely, AABBDD, AABB, AA and DD-- revealed that the *TAA1* genes are likely to exist as single copy per haploid genome. This interpretation was possible because of the choice of restriction enzymes that either did not cut within the coding sequence or cut only rarely and the use of the entire coding sequence of *TAA1a* cDNA as the probe. Despite this probe coverage, only one hybridization band

was observed in the diploids, two in the tetraploid and no more than 4 in the hexaploid (Figure 1 b). Assuming that introns of any paralogs would have caused a restriction polymorphism at this level, these results are consistent with a single copy gene per haploid genome equivalent. The presence of four bands in
5 the hexaploid blot is due to restriction within a *TAA1* gene (data not shown).

Example 4 - *TAA1 expression is confined primarily to the anther tapetum and associated with microsporogenesis - molecular biology studies*

10 The expression pattern of *TAA1* in wheat was determined by probing RNA blots with the 0.7 kb amplicon of *TAA1a*. The *TAA1a* probe strongly hybridized only to the anther mRNA, and did not show any hybridization with the root, stem, leaf, ovary or glume transcripts (Figure 2 a). For enhanced detection, RT-PCR of these RNA preparations was done with a primer pair designed to cover parts of
15 two exons with an intron in-between so as to discriminate amplicons of mRNA (~ 0.4 kb) and genomic DNA origin (~ 0.8 kb). A strong band of ~ 0.4 kb was detected in the anther sample, and there was a weak signal in the stem but not in other samples (Figure 2 b). The identity of the amplicon was confirmed by nt sequencing. Thus, *TAA1* gene expression is specific to the anther tissue.

20

Example 5 - *TAA1 expression is confined primarily to the anther tapetum and associated with microsporogenesis - In situ hybridisation studies*

In situ RNA hybridization of transverse sections of flowers at various stages of
25 development with an antisense probe (Figure 3 c and d) showed expression in the tapetum but not in ovary, epidermis, connective tissues and the filament at various ages. With a sense probe, no significant signal was detected (Figure 3 a). Generally, wheat anther development follows seven stages (Saini, 1984; Dorion et al., 1996; Lalonde et al., 1997). These stages include pre-meiosis, meiosis,
30 young microspore, vacuolated microspore (microspores irregularly shaped and in

contact with the tapetum, and microspore wall and pore formation in progress), PGM1 (microspore nucleus divides to form vegetative and generative nuclei), PGM2 (tapetal cell walls break down), and mature pollen grain. The onset of *TAA1* transcription was not evident until the microspore separation occurred at a stage corresponding to the presence of a young microspore. From then on, *TAA1* mRNA was predominantly distributed in tapetum cells and to a lesser extent in some microspores (Figure 3 b and c). *TAA1* was strongly expressed at the vacuolated microspore stage when microspore cell walls were evident. The disappearance of *TAA1* transcripts coincided with tapetum degeneration (PGM2 stage). Thus, *TAA1* transcription is confined (with the exception of weak expression in stem) to anthers, and within anthers it is localized in the tapetum from the point of the formation of young microspores to the degeneration of the tapetum (PGM2 stage).

To further test whether the *TAA1* gene product is also dominantly localized in the tapetum, the TAA1 specific poly-clonal antibodies were generated and used in *in situ* immunochemical analysis (Figure 4) of floral tissues. The results were consistent with RNA in situ hybridization, suggesting the TAA1 protein was indeed produced in the tapetum as young microspores developed to the PGM2 stage. There was also evidence of its production, albeit at a much lower level, in microspore cells at this stage. Other floral tissues did not show any distinctive signals. Taken together these data indicate that the *TAA1* gene products are associated with microspore development.

Example 6 - The *TAA1* gene product shares homology with the jojoba seed-borne FAR and the *Arabidopsis* anther-specific MS2-encoded protein

Database analysis of *TAA1* was performed to search homologous genes and explore its potential function. While BLAST searches (conducted in accordance with Altschul et al., 1997) of GenBank with the ORF of *TAA1a*, *TAA1b* and

TAA1c did not identify any significant homologs, the deduced aa sequence (>84% similarity, >74% identity among the three TAA1 peptide sequences) had homologs from *A. thaliana* (MS2 and MS2-like; Aarts et al., 1993), *Brassica napus* (B-MS2; Hodge et al., 1992) and *Simmondsia chinensis* (fatty acyl CoA reductase gene (FAR); Metz et al., 2000). *TAA1* gene products were found to be most similar to the jojoba (*S. chinensis*) FAR and the Arabidopsis putative MS2-like protein (61-65% similarity and 42-46% identity), and to a lesser extent to the MS2 and the Brassica MS2 (54-57% similarity and 35 to 39% identity). Notably, MS2 and its functional ortholog from *B. napus* (89% identical to MS2; Aarts et al., 1997) have an additional stretch of 117-aa at their amino-terminal region in comparison with TAA1, MS2-like and FAR. Of all these related gene products, only FAR has been biochemically characterized (Metz et al., 2000). Thus, even though the wheat *TAA1* gene products share a developmental connotation with the Arabidopsis *MS2* gene, TAA1 bears a greater relationship to the characterized jojoba FAR (Figure 5 a and b). The latter is associated with accumulation of storage lipids in seeds and thus presumably inconsequential to anther development. The pair-wise alignment of the aa sequence of the *TAA1a*-encoded polypeptides to that of the jojoba FAR was carried out to explore conserved domains. There are two consensus regions containing more than 12 consecutive amino acids (Fig 5 a). Interestingly, these two regions are located at the two predicted transmembrane helices (Figure 5 a) (Metz et al., 2000). Further examination of the corresponding regions on the other related gene products revealed that the first putative transmembrane helix of FAR is globally conserved while the second one is not (data not shown).

25

Example 7 - Accumulation of fatty alcohols in the *TAA1* transgenic tobacco seeds, and influence of TAA1 expression on plant phenotype

To initiate functional characterization of the *TAA1* gene *in planta*, the *TAA1a* cDNA was cloned into a binary vector under the control of a napin promoter. The

30

napin-TAA1a chimeric gene was transformed into tobacco. Tobacco seeds contain 30-43% oil and are rich in fatty acids. The potential TAA1 substrates, fatty acyl coA, are actively synthesized in the developing seeds (Frega et al., 1991). Total fatty alcohols were extracted from transgenic seeds. GC analysis on
5 fatty alcohol contents and compositions showed that the *TAA1*-encoded enzyme significantly modified the pathway of fatty alcohol synthesis in the *napin-TAA1* transgenic seeds. The amounts of the five major fatty alcohols, i.e., C18:1, C20:1, C22:1, C24:1 and C24:1 increased by 8.75%-357.47%, 57.78%-426.8.95%, 130.96%-307.72%; 145.00%-361.49% and 99.89%-5929.47%, respectively
10 (Figure 6 a).

Unexpectedly, the overexpression of the *TAA1a* gene in tobacco under the control of a 35S promoter results in significant changes to the phenotype of the corresponding transgenic plants (Figure 7). In this regard, the modified
15 transgenic plants are significantly smaller, with shorter internodes, and delayed flowering. The expression of fatty acyl Co-A reductase in these plants therefore gives rise to considerable developmental alterations in the plant. This provides evidence that changes in lipid metabolism via altered expression of *TAA1* genes can generate desirable changes to plant phenotype. Specifically, the reduction in
20 internode length and reduction in overall size of the plants will render the plants less susceptible to wind damage. Moreover, the reduction in size of the plants may permit the generation of dwarf plant specifies for horticultural purposes, and plants with increased wind resistance. The delay in flowering may also be a desirable attribute for certain horticultural situations.

25

Example 8 - Accumulation of fatty alcohols from TAA1 expression in *E. coli*

To further verify TAA1's alcohol-forming ability, a bacterial expression system was employed. Previously this approach had been originally used to determine
30 the enzymatic activity of the FAR isolated from jojoba (Metz et al., 2000). The

neutral components from bacterial cells with the control plasmid or with the plasmid containing the coding region of the *TAA1a* cDNA under the control of the *T7* promoter were subjected to GC analysis. In the control bacterial cells, there was one major peak observed. The nature of this compound was not clear.

5 In the bacterial cells expressing *TAA1a*, two additional major peaks and one additional minor peak were detected (Figure 6 b). Detention times of these three peaks were found to be identical to those of three authentic fatty alcohol standards (C14, myristi alcohol; C16:0, hexadecyl alcohol; and C18:1, oleyl alcohol). The identity of the two major peaks (C16 and C18:1) was confirmed by

10 MS-GC while the C14 fatty alcohol was not due to its very low concentration. These results suggest that TAA1 is a wheat FAR.

Example 9 - PROMOTER STUDIES - The wheat *TAA1b* promoter retains its spatial and temporal expression specificity in a distant monocot and also in a

15 *dicot*

A 1.6-Kb genomic segment upstream of the predicted start codon of the *TAA1b* was isolated by genome walking (and given GenBank accession no: AJ488930). Particle-bombardment of daylily, a distant monocot in the phylogeny of wheat,

20 with a construct containing GUS ORF immediately 3' to this fragment elaborated β glucuronidase, as shown by histochemical staining, only in the anther tapetum and microspores (Figure 8); none of the other parts such as leaves, stems, and the anther epidermis and connective tissues showed GUS expression. Furthermore, when anthers of various stages of development were bombarded, only those past

25 the tetrad formation showed GUS expression, and this result was consistent with the observations in wheat. In contrast, a CaMV 35S-GUS construct gave expression in all tissues examined. Thus, the wheat promoter is likely to be useful to manipulate male gametophyte development in other important monocot crops.

The wheat *TAA1* promoter was also found to be functional in the anthers of stably transformed tobacco (Figure 9). GUS expression was absent in other floral parts, roots and stems. In contrast, and as expected, the CaMV 35S-GUS plants showed expression in all these tissues, and an example of floral tissue is shown for comparison (Figure 9 d). While GUS activity was evident in the epidermis, connective tissues, the tapetum, microspores, and other tissues, the expression in the tapetum was not as strong as in the case of the *TAA1b*-GUS plant. Interestingly, the heterologous promoter maintained its developmental specificity in transgenic tobacco. GUS staining was not detectable in very young anthers at the time of tetrad formation (Figure 9 a). According to Koltunow et al. (1990), this would be flower buds <12 mm. In flower buds of 14 to 24 mm (Stages 2 to 6), GUS activity was detected in the tapetum, and in buds >15 mm (Stage 4), a strong GUS staining was also seen in microspores (Figure 9 b and c). Collectively, these results show cross-functionality of any *cis* elements of the *TAA1* promoter with *trans*-acting factors in daylily and tobacco.

Experimental procedures

Plant materials, DNA and RNA isolation

Hexaploid spring wheat (*Triticum aestivum* L. cv. Karma, genomic complement AABBDD), tetraploid wheat (*Triticum turgidum* L. cv. Sceptre, genomic complement AABB), and two diploid wheat species (*Triticum urartu*, ssp. Nigrum, genomic complement AA; *Aegilops squarrosa*, ssp. Tauschii, genomic complement DD) were grown in an open field or in a greenhouse under standard conditions. Five-week-old seedlings were used for DNA isolation and for leaf, root and stem RNA isolation. Anther, ovary, glume and pilea tissues were collected 1-3 days prior to anthesis for RNA purification. DNA extraction was carried out following the published protocol (Wang et al., 1998). Total RNA was

extracted using Trizol™ reagent (Life Technologies/Gibco-BRL, Burlington, Ontario) following the supplier's recommendations.

RT-PCR, 5'- and 3'-RACE, and other RNA and DNA technologies

5

The nucleotide acid-related enzymes used were from Life Technologies (Burlington, Canada) except otherwise stated. Five µg of total RNA derived from various tissues was used to synthesize the first strand cDNA by reverse transcriptase using primers OL2707 (5'GACTACGTCGTCCAAGGCCG3' -
 10 SEQ ID NO: 10) and OL2708 (5'GTCGAACTGCTTGAGCAG CGC3' - SEQ ID NO: 11). The PCR reactions were carried out with a Techne Genius DNA thermal cycler (Duxford, Cambridge, UK) under the following conditions: 94°C for 1 min, 43°C for 1 min, 72°C for 2 min, 35 cycles, followed by 10 min's incubation at 72°C. The amplified products were subjected to DNA sequencing.
 15 Based on obtained sequences, primers OL2881 (5'GCAGAACCTGACATACTTC3' - SEQ ID NO: 12) and OL2885 (5'GAGGCGGTACCT GAGCAT3' - SEQ ID NO: 13) were designed for RT-PCR detection and genomic DNA amplification.

20 Antisense primers OL2884 (5'TTCGCATAGCCGATCACG3' - SEQ ID NO: 14) and OL2883 (AATGCCGGCCCTGGTAAG3' - SEQ ID NO: 15) and sense primers OL2880 (5'CAGGTGGCCAAA CACATA SEQ ID NO: 16 and OL2881 (5'GCAGAACCTGACATACTTC - SEQ ID NO: 12) were designed for 5'- and 3'-RACE which were conducted using 5' and 3' RACE kits (Life Technologies,
 25 Burlington, Ontario) following the manufacturer's protocol. The resulting cDNA fragments were either directly subjected to DNA sequencing or cloned into a T/A vector using the Original TA Cloning Kit™ (Invitrogen, Carlsbad, CA) for sequencing.

RNA and DNA gel blot analyses were performed essentially as described (Nair et al., 2000). The predicted coding region of *TAA1a* was used as a probe.

5 *cDNA library construction and screening*

Wheat anther poly(A)⁺ RNA was isolated using an mRNA kit (Clontech, Palo Alto, CA). Approximately 5 µg poly(A)⁺ RNA was used for cDNA library construction using a ZAP cDNA Gigapack III Gold Cloning Kit™ according to
10 the supplier's instructions (Stratagene, La Jolla, CA). The ligated vector was packaged into phage particles using Gigapack III gold packaging extracts (Stratagene, La Jolla, CA). A total of 2.2×10^7 primary pfu were obtained. Library screening was conducted using the 5' RACE PCR cDNA fragment as a probe to hybridize phage plaques containing approximately 250, 000 recombinant
15 clones. The positive plaques were isolated and the phagemids were excised *in vivo* from the Uni-ZAP XR™ vector using the ExAssist/SOLR™ system (Stratagene, La Jolla, CA). The inserted cDNA sequences in the purified phagemids were determined by DNA sequencing.

20 *Production of polyclonal antibodies against TAA1*

The entire coding region of *TAA1a* was directionally cloned in-frame into the *Bam*H1-*Eco*RI sites of plasmid pRSET A (Invitrogen, Carlsbad, California) to make plasmid pTAA238. Fusion protein was expressed in *E. coli* strain
25 BL21(DE3)pLysS (Invitrogen). The TAA1 fusion protein was purified and injected into rabbits following the procedures (Wang et al., 1999). Polyclonal antibodies were harvested and purified as described (Wang et al., 1999).

In situ RNA hybridization and immuno-cytolocalization

30

Plant materials were infiltrated overnight at 4°C in 4% paraformaldehyde (PFA) with a 100mM phosphate buffer pH 7.2. The fixed material were dehydrated in a graded ethanol series and then embedded in paraffin (Paraplast plus x-tra). Sections were cut at 8 µm thickness and mounted on glass slides (Superfrost™ plus, Fisher Scientific, Nepean, Ontario). For *in situ* RNA hybridization, probes were prepared using MAXIscript™ *in vitro* transcription kit and BrightStar™ P soralen-Biotin nonisotopic labeling kit (Ambion, Austin, Texas) according to manufacture's protocols. A DNA fragment of 550 bp of the *TAA1a* cDNA starting from the predicted start codon was directionally cloned into pBluescript™ II KS⁺ phagemid vector (Stratagene, La Jolla, CA) at the *Bam*HI-*Xho*I sites to produce plasmid pTAA253. The antisense transcripts synthesized *in vitro* by T3 polymerase using *Xba*I-linearized plasmid pTAA253 as a template were used to detect the *TAA1 mRNA*. The partial *TAA1* sense transcripts generated by T7 polymerase using plasmid *Xho*I-linearized plasmid pTAA253 as a template were served as a control. *In situ* hybridization was carried out essentially following the instructions of the mRNA locator-Hyb™ kit (Ambion).

For *in situ* immunological detection, slides mounted with fixed sections of wheat florets are incubated in the blocking solution containing 1:1000 TAA1a immune or pre-immune serum for overnight at 4 °C. Visualization of immuno-reaction was as described (Cho and Kende, 1998).

Vector construction and genetic transformation

Plasmid pRD400 (Datla et al., 1992) was modified by flipping-over the region containing the polylinker and the NPT II gene cassette to generate a binary transformation vector pAMW281. Two pieces of DNA fragments including a 2.4 kb fragment containing a CaMV 35S promoter and a uidA gene from plasmid pRD410 (Datla et al., 1992) digested with *Hind*III and *Eco*RI, and a 0.7 kb fragment containing a CaMV 35S terminator from plasmid pHS724 restricted

with *EcoRI* and *KpnI* (Huang et al., 2000) were co-ligated into the backbone of pAMW281 digested with *HindIII* and *KpnI* to produce plasmid pAMW287. A 1.4 kb *napin* promoter obtained from digestion of plasmid pJOY43 with *HindIII*-*BamHI* (Nair et al., 2000) and the 1.4 kb *TAA1a* entire coding region resulting from plasmid pTAA238 restricted with *BamHI* and *EcoRI* were co-ligated into the *HindIII*-*EcoRI* sites of plasmid pAMW287. The resulting plasmid pAMW 458 consisted of the *Napin* promoter, the *TAA1a* coding region and the 35S transcription terminator.

10 *Agrobacterium*-mediated transformation was employed for production of tobacco (*Nicotiana tabacum* cv Xanthi) transgenic plants using published protocols (Huang et al., 2000). The presence of foreign genes in independently derived kanamycin-resistant cell lines was confirmed by PCR and Southern blot analyses, according to standard techniques.

15

Gas chromatography (GC) analysis

For plant GC analysis, mature seeds were harvested from TAA1 transgenic plants, non-transgenic wild-type control plants and control transgenic plants. 20 Seed samples were ground and saponified with 10% potassium hydroxide dissolved in methanol with 1% water, incubated at 80 °C for 2 hours. The mixture was then extracted twice with hexane and subjected to GS analyses for total fatty alcohol and fatty acid contents and compositions as described by Katavic et al. (1995). Analysis was done on a 30 M DB-5 column starting at 250 °C to 300 °C at 25 5 °C/min. The identity of the fatty alcohol peaks was based on retention times of authentic fatty alcohol standards and confirmed by GC-MS. The relative amount of fatty alcohols was calculated on the basis of fresh weight of the seeds and normalized according to internal contents of β -sitosterol extracted in the same procedure.

30

For *E. coli* GC analyses, 200 ml bacterial cells with appropriate plasmids were grown to OD value of 0.5 at 30 °C. After addition of IPTG (0.2mM), the culture was allowed to grow for 3 hr. The bacterial cells were harvested. Subsequent extraction and GC analysis were essentially as above. Qualification of fatty alcohols was based on flame ionization detector peak areas, which were converted to mass units by comparison with an internal standard which was added before the extraction.

TAA1 promoter isolation and analysis

The upstream regulation region of *TAA1b* was isolated from the hexaploid spring wheat cultivar Karma (genetic complements: AABBDD) using a Universal GenomeWalker™ Kit (Clontech, Palo Alto, CA). The resulting 1.7 kb DNA fragment was cloned into a T/A vector (Original TA Cloning Kit, Invitrogen, Carlsbad, CA) for further analysis.

Plasmid pRD400 (Datla et al., 1992) was modified by flipping-over the region containing the polylinker and the NPT II gene cassette to generate a binary transformation vector pAMW281. Two pieces of DNA fragments including a 2.4 kb fragment containing a CaMV 35S promoter and a uidA gene from plasmid pRD410 (Datla et al., 1992) digested with *Hind*III and *Eco*RI, and a 0.7 kb fragment containing a CaMV 35S terminator from plasmid pHS724 restricted with *Eco*RI and *Kpn*I (Huang et al., 2000) were co-ligated into the backbone of pAMW281 digested with *Hind*III and *Kpn*I to produce plasmid pAMW287, consisting of the 35S-GUS-PolyA cassette. Plasmid pAMW445 containing *TAA1b* promoter-GUS-PolyA was obtained by cloning the isolated 1.5 kb *TAA1b* promoter into the *Hind*III-*Bam*HI sites of plasmid pAMW287. *Agrobacterium*-mediated transformation was employed for production of tobacco (*Nicotiana tabacum* cv Xanthi) transgenic plants using published protocols (Huang et al., 2000). Presence of foreign genes in independently derived kanamycin-resistant cell lines was confirmed by PCR and Southern blot analyses.

For transient expression analysis, microprojectiles coated with *35S/GUS* or *TAA1* promoter/GUS chimeric genes were bombarded into the transverse sections of flowers of a monocotyledonous plant species, daylily (*Hemerocallis*
5 *lilioasphodelus*) essentially as described (Chen et al., 1998).

To analyze GUS expression in transgenic plants, the flower buds were collected from the primary transgenic tobacco plants (F_0). Anthers were cut transversely and incubated in a GUS-assay buffer (0.1 M phosphate buffer pH7.0, 2 mM
10 $K_3[Fe(CN)_6]$, 2 mM $K_4[Fe(CN)_6]$, 1 mM EDTA, 0.1% Triton) with 1 mM X-Gluc (5-bromo-4-chloro-3-indoyl- β -D-glucuronide) overnight at 37°C. After incubation, the anthers were observed under a microscope. Typical anthers were embedded in paraffin and then sectioned in 6 μ m thickness for the further observation. The histochemical assay on the daylily flowers was performed 24 h
15 post bombardment essentially as described (Wang et al., 1998).

Whilst the present invention has been described with particular reference to specific examples and techniques, the invention is not intended to be limited in
20 this regard, and numerous peptide and nucleotide sequences, constructs, transformed organisms, methods, and products that are not directly described herein are intended to be encompassed within the scope of the present invention.

Sequence listing

SEQ ID NO:1 (TAA1a cDNA sequence)

5 (Putative start and stop codons are in bold/underlined)

CTCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCGCACGCACGCACGCACGCACACAAGAAAAAATCAAGATGGTTGACAC
 ACTGAGTGAAGAGAACATCATTGGATACTTCAAGAACAAGAGCATCCTCATCACTGGATCAACAGGCTTTCTTGGAAGA
 TACTGGTGGAGAAGATACTGAGAGTTCAACCTGATGTGAAGAAGATCTACCTCCCGGTGCGAGCGGTGGATGCCGCGGCG
 10 GCAAAGCATCGGGTGGAGACTGAGGTGGTAGGGAAGGAGTTGTTCTGGGCTTCTGAGGGAGAAGCACGGGGGCAGGTTTCA
 ATCTTTCATCTGGGAAAAGATCGTCCCATTTGGCCGGAGACGTGATGCGCGAGGACTTCGGCGTCGACAGCGAGACCTGA
 GGGAGCTCCGGGTGACCCAGGAGCTCGATGTCATCGTTAATGGCGCCGCCACCACCAACTTCTACGAAAGGTATGATGTG
 GCTCTAGACGTGAACGTGATGGGAGTGAAGCATATGTGCAACTTCGCCAAGAAGTGCCCCAATCTCAAGGTGCTCCTCCA
 15 TGTCTCCACGGCTTACGTGGCGGGTGAGAAGCAAGGGCTGGTGCAAGAGAGACCATTCAAGAATGGCGAGACGCTGCTCG
 AGGGGACCCGCTCGACATCGACACTGAGCTGAAACTGGCCAAGGACCTGAAAAAGCAGCTTGAGGCCGACGTTGATTTCG
 TCGCCCAAGGCCGAAAGGAAGGCCATGAAGGATCTTGGCCTTACCAGGGCCCCGCACTTCAGGTGGCCAAACACATACGT
 GTTCACCAAGTCGATGGGGGAGATGGTGCTAAGCCAGTTGCAGTGTGATGTCCCCGTTGTTCATCGTCCGTCCCAGCATCA
 TCACAAGTGTCCAGAACGACCCACTGCCCGGATGGATCGAAGGCACCAGGACGATCGACACGATCGTGATCGGGCTATGCG
 20 AAGCAGAACCTGACATACTTCTTGCCGACCTCAACCTCACCATGGATGTGATGCCGGGCGACATGGTGGTGAATGCGAT
 GATGGCGGCAATAGTGGCACACAGCTCGTCCTCATTGGAGAAGACAAAGTCACATCCCAAGCAACATGCACCGGCGGTGT
 ACCACGTGAGCTCGTCGCTGCGTAATCCGGCACCATAACAATGTGCTTCATGAGGCTGGGTTTCGGTACTTCACGGAGCAC
 CCTCGCGTGGGCCCTGACGGTCCGACCGTGCCTACCCATAAGATGACATTCTCAGCAGCATGGCTTCCTTCCACCTATT
 TATGATGCTCAGGTACCGCCTCCTCCTCGAGCTCCTCCACCTGCTCTCCATCCTCTGCTGCGGCCCTCTTCGGCCTCGACA
 25 CCGTCTACCACGACCAAGCACGCAAGTACAGGTTCTGTATGCACCTGGTGGATCTGTACGGGCCCTTTGCGCTGTTCAAG
 GGGTGCTTCGATGACGTCAACCTAAACAAGCTCAGGCTCGCCATGACCAGCAACCATGGTAGCCTCTTCAATTTGACCC
 GAAGACCATTGATTGGGACGAGTACTTCTACAGGGTCCACATCCCCGGGGTCATAAAGTACATGCTCAAGTGAATATCC
 GTGCACCGAAATTCAGGCGTTGCCTTAATAATTAATAATCGTACGATTGTAAGAAATGTCCTCCCAAATTGGGTTCTAA
 CCGTTAAATAATCTATATGAAAGAGGAAAATGTGTCTGTGCGCTTGATAGAGCAGGCATCAACGTTGCATAAGTTTCTTGA
 30 AGACAAGGAATGCTACATTATGTAGTCCCATTTCTGGTTTCATGTATTGTATTGCTAATTATGACTAGTATTGATTGTTGTT
 ACCAAGCCGAATTCCAGCACACTGGCGGCCGTTACTAGTGGATCCGAGCTCGGTACCAAAAAAAAAAAAAAAAAAAAAA

35

40

45

50

55

60

65

70

75

SEQ ID NO:3 (TAA1b cDNA sequence)

(Putative start and stop codons are in bold/underlined)

5 CTCTCTTCTTCCGTCTCTCATTCTCTCCTCCCGAGCTTCATAGCTCACACGCACACAAAAACCAAGGCAAGATGGTGG
GCACGCTGGATGAGGGGAAGATCGTCGACTACTTCAGGAACAAGAGCGTGCTCATCACCGGAGCCACGGGATTCCTTGGC
AAGATAATGGTGGAGAAGATCCTGCGGGTCCAGCCGGACGTGAAGAGGATCTACCTGCCGGTGCGGGCGGCCGACGCCGC
GGCGGGCAGGCGCCGGGTGGAGACCGAGGTGGTGGGGAAGGAGCTGTTCTGCGTGCTGCGGGAGCGCCACGGCGCCGGGT
TCGACGCCTTCGTGCGCCGACAAGGTGGTGGGGCTGGCCGGCGACGTCATGCGCGAGGGCTTCGGCGTCCGACCCCGCCACG
10 CTGCGGGACCTCCGGCTCGCCGACGAGCTCAACGTCACTCGTCAACGGCGCCGCCACCACCAACTTCTACGAAAGGTACGA
CGTGGCCCTGGACGTGAACGTGGTGGGGGTGAAGCACATGTGCGACTTCGCCCCGAGGTGCCCCAACCTCGAGGTGCTCA
TGCACGTCTCCACGGCCTACGTGCGCCGGCGAGAAGCAGGGGGCTGGTTCCGGAGAGGCCGTTCAAGGACGGCGAGACGCTG
CGCGACGACGGCACCCAACTCGACATCGACGCCGAGATGAGGCTGGCCAAGGACCTCAGGAAGCAGATGGAGGCCGACGA
CGATGTGGACCCCAAGGCCAGAGGAAGGCCATGAAGGACCTCGGCCTCACCAGGGCCAGGCACTTTGGGTGGCCCAACA
15 CGTACGTGTTACCAAATCCATGGGGGAGATGATGCTGGCCAGATGATGCGCGGGGGCGACGTGCCCCGTCTCATCGTC
CGGCCCAGCATCATCACAGCGTCCAGAACGACCCACTGCCAGGATGGATCGAAGGCACCAGGACGATCGACGCAATCCT
GATCGGGTACGCGAAGCAGAGCCTGTCGTGCTTCCTCGCCGACCTCGACCTAACCATGGACGTGATGCCCCGGCGACATGG
TGGTGAACGCGATGATGGCGGCCACGTTGGCACATGCCTCCTCCACTCAGACATCAGAGCCAGAGAAGAAGCCGCCCTCCG
CAGCAGCAACACCCTCACTCGGTGCCGGCAGCGCCAACGGTGTACACGTGAGCTCGTCGCTGCGGCACCCGGCTCCGTA
20 CGCGGTGTTGTACCGAACGGGGATCCGGTACTTCGAGGAGCACCCACGGGTGGGGCCTGATGGCCGCCCCGTGCGCACCC
GTAAGGTGCGGTTCTCGGCAGCATCGCGGCGTTCCACCTATTATGGTGCTCAAGTACCGTGTCCCCCTTGAATCCTC
CGCCTGCTCTCCATCCTCTGTTGCGGGCCTCTTTGGCCTTGCCGCCCTCTACCACGACCTCGCCCGCAAGTACAGGTTCTG
GATGCAGCTGGTGGACCTGTACGGGGCCCTTCTCGCTCTTCAAGGGTTGCTTCGACGATGTAAACCTCAACAAGCTCAGGC
TCGCCATGGCCGACGGTGACCATGCCGATTCCGCATTCAACTTTGACCCCAAGACCATTGACTGGGACGACTACTTCTTC
25 AAGGTCCACATCCCTGGTGTATGAAGTACGTCCACAAGTGAATGTTCTGTGTGCGATCTGCTTCTGCGTGCTGAGAAGGA
ATGGAGGAAATCAAATTAATGGTAGCGCTAGTGTGCCCTTGCTTGTGTGTGTAACCTCCTTCTTCGTTTCATCGAGTATTA
TTGGTTGAGTATTGATTGTATTGTTCATTGGAAGTTAAATTAACAGTACTATGAGTATAACTAAGATGAAATTACTTGC
ATCATGGCGGGTCTCTAAACTAAGATAGTACAAGGATCCTATGAAGTACATTGAAATTACTTAGTACTTTTCATGGTAC
TATCATAATACAAAAAAAAAAAAAAAAAAAAA
30
35
40
45
50
55
60
65
70
75

SEQ ID NO:5 (TAA1c cDNA sequence)
(Putative start and stop codons are in bold/underlined)

[illegible]

30

35

40

45

SEQ ID NO: 7 (TAA1a genomic sequence)

(introns are underlined, transcription start site and start and stop codons are indicated in bold/underlined)

5 CCTCTTTAAGTCGGTCAGGTACGTAGCAAATAAACCAAACAAGCAGGACATGTTTGAAAATCACTGTTCTAACTTTAATA
 ATGCACACAAGGAATTGAAGGCTTGATAAATCCTGTGCTAGTACCACCCATCCGACTGTCGGTTTTTAAGACATGGTAGA
 GTAGAGTACTAACATAACGATCTTCAAGATATTGTGTCCAAGTTCCTTGCCACAGGTTTGTGCTATTGAGCCAAAATATA
 10 CCACTGAATTATGTGCTAATAGTGTAAATTATTGGTCAATTTTTTGTCTAACGAGAGAAAAATACGCTATGCGTTTTGAA
 GGGATGGAGTACGATGATACTACTCTATTGTACTCCACGGATCAATTGTAGGAGTACCCTCTAATTTCTAATGGTATAAC
 TTTGATGACATAACGATTTGTATTACGTTGGTTGAAATTGTACATGGGCTTCTATAAAGCGAAAAAGAAAGGAATACTGAG
 CTTCTCTAGTTTTCTATAAMCCAGAGTTAGACGTCTACTGGACAACCTCATTCTAAGTGTGTAGCAATAATACTGAGTA
 CTACCGTTGTCCACTTGAGCTATTAATTTGATGGCTAGCAACGTACTGCAACCCCAATAATAACCTGTTTGAAAATCT
 15 AGAGTACCTCTACGCTTATTTTGACTGCATGTGATTCCTCTCATGATCACCAGGTCCCTTGKTTTGGGTTGGACAGCGC
 ATAAATGTTGCGCTCAAGTGCCTCAAATTAAGACTCCCTCTCTTACCTACAACACAAAAGACCTTTTTTTTCAGCAAAAG
 AGGGCGCCACCCCTCCGATTTTCATATAATGAAACCAACTGGTTCAACTGCCAAACAAACAAACAAACAAACGAAACA
 CCTTCATGGCCCGTGTATAAATATTGTGTACATGACCTCTTTCCACTCACCCACACAAATCATCTCCTCTCTCTCTC
 TCTCTCTCTCTCTCTCGCACGCACGCACGCACGCACACAAGAAAAAATCAAGATGGTTGACACACTGAGTGAAGAGAAC
 20 ATCATTGGATACTTCAAGAACAAGAGCATCTCATCACTGGATCAACAGGCTTTCTTGGAAGAGTATGCATGCATGCAT
 AATGTTTATGCACACATATTACGCATGCATATACTTTTTTTTTGGCAAATTCTAGAAGTTTTTTGCGTCAAGTTTCATATGT
 GCTGTCTCATCTGTTAATTAGTTCACCTGTTGGTGTGCGTATATGCAGTACTGGTGGAGAAGATACTGAGAGTTCAACCT
 GATGTGAAGAAGATCTACCTCCCGGTGCGAGCGGTGGATGCCGCGCGGCAAGCATCGGGTGGAGACTGAGGTAGTGT
 GTCCATCATGGTTCTTCATGATCTATACCCCTTTACCCCTCTCATGATTCCTTTTGATCCCTTTTCTTCAGAGTTAGCGCTG
 25 ACTAATTTCTTTTCTATATGCGCAGGTGGTAGGGAAGGAGTTGTTCCGGCTTCTGAGGGAGAAGCACGGGGGAGGTTTC
 AATCTTTCATCTGGGAAAAGATCGTCCCATTTGGCCGAGACGTGATGCGCGAGGACTTCGGCGTGCACAGCGAGACCTTG
 AGGGAGCTCCGGGTGACCCAGGAGCTCGATGTCTGTTAATGGCGCCGCCACCACCAACTTCTACGAAAGGTGCGTCA
 TGTCAACTGATTGTTTGTCCAAGAAAGGAAAAATCAGCAGAGAACTTGAGTAGGTGCAGCAAGAGTACGTACGCAGTGA
 ATGGTCTCAGAAGAACACTTGTGTGCGTGCAGGTATGATGTGGCTCTAGACGTGAACGTGATGGGAGTGAAGCATATGTG
 30 CAACTTCGCCAAGAAGTGCCCAATCTCAAGGTGCTCCTCCATGTCTCCACGGGTACGTACACCAACTCTACAGGTAAAA
 ACAAAAATAAAGTTCTTGATGTTAATTATAATACACCTAGATTTGATTTACAAATGAAGTTAATAAATTCATATATGAG
 TTGGTGCAGCTTACGTGGCGGGTGAGAAGCAAGGGCTGGTGAAGAGAGACCATTCAAGAATGGCGAGACGCTGCTCGAG
 GGGACCCGCCTCGACATCGACACTGAGCTGAAACTGGCCAAGGACCTGAAAAAGCAGCTTGAGGCCGACGTTGATTCGTC
 GCCCAAGGCCGAAAGGAAGGCCATGAAGGATCTTGCCCTTACCAGGGCCCGGCACTTCAGGTGGCCAAACACATACGTGT
 35 TCACCAAGTCGATGGGGGAGATGGTGCTAAGCCAGTTGCAGTGTGATGTCCCCGTGTCATCGTCCGTCCCAGCATCATC
 ACAAGTGTCCAGAACGACCCACTGCCCGGATGGATCGAAGGCACCAGGTTTATTATATGTTTCTTGTCTCTTCTGCTC
 CCAAATTTAGAGTGCAATCGTCTTACTCTGTTGCAAATGCCAAAAGAAGTAAATATGATATTTGTTCAATGTAAAAATG
 TAAATTGCAGGACGATCGACACGATCGTGATCGGCTATGCGAAGCAGAACCTGACATACTTCTTGCCGACCTCAACCTC
 40 ACCATGGATGTGGTAAGCAACGTTGTACTATGCATGCAGTTAAGATATATTCCAGGCAATGGTTGGTTGTCAGTCCAGTC
 CAGGAATCCGTACAGTAAGATGAATTTGACGACGATGGTGAAAGCAATCGTTTGGTTGGGTATATGTTGCTGTAGATGC
 CGGGCGACATGGTGGTGAATGCGATGATGGCGGCAATAGTGGCACACAGCTCGTCCTCATTGGAGAAGACAAAGTCACAT
 CCCAAGCAACATGCACCGGCGGTGTACCACGTGAGCTCGTCTGCTGCGTAATCCGGCACCATACAATGTGCTTCATGAGGC
 TGGGTTTCGGTACTTCACGGAGCACCTCGCGTGGGCCCTGACGGTGCACCGTGCCTACCCATAAGATGACATTCCTCA
 45 GCAGCATGGCTTCCTTCCACCTATTTATGATGCTCAGGTACCGCTCCTCCTCGAGCTCCTCCACCTGCTCTCCATCCTC
 TGCTGCGGCTCTTCGGCTCGACACCTCTACCACGACCAAGCAGCAAGTACAGGTTAGTTAGTTGGTTGAAATCTTG
 TGCGGTGTATCTTCTTGATGGCTCCACATAATTAAGATGACACGACTTTTATTGTTGTATTGTTATAGGTTCTGTATG
 CACCTGGTGGATCTGTACGGGCCCTTTGCGCTGTTCAAGGGGTGCTTCGATGACGTCAACCTAAACAAGCTCAGGCTCGC
 CATGACCAGCAACCATGGTAGCCTCTTCAATTTGACCCGAAGACCATTGATTGGGACGAGTACTTCTACAGGGTCCACA
 TCCCCGGGGTCATAAAGTACATGCTCAAGTGA

CTCTTCAAGGGTTGCTTCGACGATGTAAACCTCAACAAGCTCAGGCTCGCCATGGCCGACGGTGACCATGCCGATTCCGC
ATTCAACTTTGACCCCAAGACCATTGACTGGGACGACTACTTCTTCAAGGTCCACATCCCTGGTGTGATGAAGTACGTCC
ACAAGTGA

SEQ ID NO: 9 (TAA1c genomic sequence)

(introns are underlined, transcription start site and start and stop codons are indicated in bold/underlined)

5 ACTCCGACACAATATTGACTGCTGATGTTCTCTTATGATCACTGAGTCTTTGTTTCGGTTGGACAGCGCATAAATATG
TTGCGCTCAAGTGCCTCAAATTAAGACACCCCTCTTTACTACACACGAAAGACCTTTTTCTTAGCAAAAATTTTCA
TACAATGAAACCAACTGGTTCAACTGCCAAAACAACAGACTCCAAGACAAAACACCTTGATGGCCCGTGTATAAATATTG
TGTACAGGAGCCTCTTTCCACTCACCTACACAAATCATCTCCTTCTTCTCTCTCTCTCTCTCCCTGCTCTCCCTGCT
10 CTCTCTCTCTCTCTCTCTCTCTCTCTTGGCGACACAAGAAAAAAATCAAGATGGTTGACACACTGAGTGAAGAGAAGATCA
TTGGATCACTTCAAGAACAAGAGCATCCTCATCACTGGATCAACAGGCTTTCTTGGAAGAGTATGCATGCATGCACGCAT
AATGTTTATCAAAACATATTACGCATGCATATACTATTGTTGCAAATCTAGAAAGTTTTTTTTTGGCTCAAGTTGATA
TGTGTTGTGCTCATCTGTTAATTAGTTCTACTGTTGGTGATATGTGCAGTACTAGTGGAGAAGATACTGAGAGTTCAAC
CTGATGTAAAGAAGATCTATCTCCCGGTGCGAGCGGTGGATGCCGCGCGCGGAAGGATCGGGTGGAGACTGAGGTATGTG
TTGTCCATCATGGTTCTTCATGATCTAGACCTCTACTGCTCTCATGATTCTTTTGATCCCTTTGCTTGAGTGTGGTACT
15 GAATAATTTATTTTCTATGTGCCAGGTGGTAGGGAAGGAGTTGTTCGGGCTTCTGAGGGAGAAGCACGGGGACTGGTTT
CAATCTTTTCATCTGTGAAAAGATCGTCCCATTTGGCCGGAGATGTGATGCGTGAGGACTTTGGCGTGCACAGCGAGACCT
GAGGGAGCTCCGGGTGACCCAGGAGCTCGATGTCATCGTTAATGGCGCCGCCACCACCAACTTCTACGAAAAGGTGCGTCCG
TCGTCAACTGATTGTTTTGTTGCCAAGAAAGAAAAAATCAGCAGAGAACTTGAGTAGGTGCAGCAAGAGTACGGAGTGG
AATTGTGTGCGTGCAGGTATGATGTGGCCCTGGACGTGAACGTGATGGGAGTGAAGCATATGTGCAACTTGCCCAAGAAG
20 TGCCCAATCTCAAGGTGCTCCTCCATGTCTCCACGGGTACGTACACCAACTCTACAAATTACCATCATGGACTATGAAC
TTGGATGCTTCTGGGGAAAAACAAAATGAAGTTCTTGGATGTAATTAAAGTACACCTAGATTTGATTTACAAATCAAGTT
AATGAATTCATACATGAGTTGGTGCAGCTTATGTTGCGGGTGAGAAGCAAGGACTCGTGCAAGAGAGACCATTCAGAAT
GGCGAGACGCTGCTCGAGGGGACCCACCTCGATATCGACACCGAGCTGAAACTGGCCAAGGACCTGAAAAAGCAGCTTGA
25 GGCCGACGCCGACTCGTCGCCCAAGTCCCAAAGGAAGGCCATGAAGGACCTTGGGCATACCAGGGCCCCGGCACTTCGGGT
GGCCGAACACATACGTGTTCAACAAGTCGATGGGGGAGATGGTGCTTGGGCCAGTTGAAGTGTGATCTCCTCTGTTGTCATC
TCCGCTCCAGCATCATCACCAGTGTCCGAGACGCCACTGCTCCGGATGATCGAAGGCACCAAGGTTCCGTTATATGCTT
CTCTTTCCCTTCCCTGCTCTAAATTTAAAGTGCAATCGTTTAAATCTGTTGCAAATGCAAGTAAATAAAGGATGTTTGT
TCAATGTAAAAATGTAAATTGCAGGACGATCGACACGATCGTGATCGGCTATGCGAAGCAGAACCTGCATACTTCTTGG
30 CGGACCTCAACCTCACCATGGATGTGGTAAGCAACGTTGCACTATGCATGCAGTTAATTAACATATATTCCAGGCATGCA
ATGGTTGGTTGTCAGTCCAGGAATCCATACAGTAAGATATGGATTTCAACGATGGCGGTGAATGCAATCGTGTGGTTGGG
TATATGTTGGTGCAGATGCCGGGCGACATGGTGGTGAATGCGATGATGGCTGCCATCGTGGCGCACAGCTCGTCCTTATT
GGAGAAGACACAGTCACATCCCGAGCCACACGCCGGGTGTACCACTGAGCTCGTGCGGCGGTAAACCGGCCGCT
35 ACAATGTGCTGCACGAGGCTGGGTTTCGGTACTTACAGGAGCACCTCGGTTGGGCCCTGCAGCGGCCGACCGTGCAC
CATAAGATGACATTCCCTCAGCAGCATGGCTTCCTTCCACCTCTTTATGATGCTCAGGTACCGCCTCCTCCTTGAGCTCCT
CCACCTGCTCTCCGTCTCTGTTGTGGCCTCTTCGGCCTCGACACCCTCTACCACGACCAAGCACGCAAGTACAGGTTAG
TCGGTTTAAATCTTTTTCGGATGGCATTTTTGATAACAAGTATTTCCGGACGGAGGGAGTATCTTCTTGATGGCGCGGCA
40 TATATGATGACACGGCTTTATTGTTATATTGTTGTAGGTTTCGTGATGCACCTGGTGGATCTGTATGGGCCCTTCGCGCT
GTTCAAGGGCTGCTTCGATGACGTCAACCTAAACAAGCTCAGGCTCGCCATGACCAGCAACCATGGAAGCCTCTTTAATT
TCGACCCCAAGACCATTGACTGGGACGATTACTTCTACAGCGTCCACATCCCCGGGGTCTTAAAGCACATGCTCAACTGA

45

50

55

SEQ ID NO: 2

TAA1a amino acid sequence

5 MVDTLSEENIIGYFKNKSILITGSTGFLGKILVEKILRVQPDVKKIYLPVRAVDAAAACHRVETE VVGKELFGLLREKHG
GRFQSFIEWEKIVPLAGDVMREDFGVDSETLRELRTQELDVIVNGAATTNFYERYDVALDVNVMGVKHM CNFAKKCPNLK
VLLHVSTAYVAGEKQGLVQERPFKNGETLLEGTRLDIDTELKLA KDLKKQLEADV DSSPKAERKAMKDLGLTRARHFRWP
NTYVFTKSMGEMVLSQLQCDVPVVIVRPSIITSVQNDPLPGWIEGTRTIDTIVIGYAKQNLTYFLADLNLTMDVMPGDMV
10 VNAMMAAIVAHSSSSLEKTKSHPKQHAPAVYHVSSSLRNPAPYNVLHEAGFRYFTEHPRVGP DGRTVRTHKMTFLSSMAS
FHLFMMLRYRLLELLHLLSILCCGLFGLDTLYHDQARKYRFVMHLVDLYGPFALFKGCFDDVNLNKLRLAMTSNHGSLF
NFDPKTIDWDEYFYRVHIPGVIKYMLK

15

20

25

30

35

40

45

50

55

60

65

70

75

SEQ ID NO: 4

TAA1b amino acid sequence

5 MVGTLDEGKIVDYFRNKSVLITGATGFLGKIMVEKILRVQPDVKRIYLPVRAADAAAARRRVETE VVGKELFCVLRERHG
AGFDAFVADKVVGLAGDVMREGFGVDPATLRDLRLADELNVIVNGAATTNFYERYDVALDVNVVGVKHMCDFARRCPNLE
VLMHVSTAYVAGEKQGLVPERPFRDGETLRDDGTQLDIDAEMRLAKDLRKQMEADDDVDPKAQRKAMKDLGLTRARHFGW
PNTYVFTKSMGEMMLAQMMRGGDVPVVI VRPSIITSVQNDPLPGWIEGTRTIDAILIGYAKQSLSCFLADLDLTMDVM PG
10 DMVVNAMMAATVAHASSTQTSEPEKKPPPQQQHPHSVPAAPT VYHVSSSLRHPAPYAVLYRTGIRYFEEHPRVGP DGRP V
RTRKVRFLGSIAAFHLFMVLKYRVPLELLRLLSILCCGLFGLAALYHDLARKYRFVMQLVDLYGPFSLFKGCFDDVNLNK
LRLAMADGDHADSAFNFDPKTIDWDDYFFKVH I PGVMKYVHK

15

20

25

30

35

40

45

50

55

60

65

70

75

SEQ ID NO: 6

TAA1c amino acid sequence

5 MVDTLSEEKIIGYFKNKSILITGSTGFLGKILVEKILRVQPDVKKIYLPVRAVDAAAAKDRVETEVVGKELFGLLREKHG
DWFQSFICEKIVPLAGDVMREDFGVDSETLREL RVTQELDVI VNGAATTNFYERYDVALDVNVMGVKHM CNFAKKCPNLK
VLLHVSTAYVAGEKQGLVQERPFKNGETLLEGTHLDIDTELKLAKDLKKQLEADADSSPKSQRKAMKDLGITRARHFGWP
10 NTYVFTKSMGEMVLGQLKCDLPVVIVRPSIITSVQNDPLPGWIEGTRTIDTIVIGYAKQNLTYFLADLNLTM DVM PGDMV
VNAMMAAIVAHSSSLEKTQSHPEPHAPAVYHVSSSRNPAPYNVLHEAGFRYFTEHPRVGP DGRTV RTHKMTFLSSMAS
FHLFMMLRYRLLELLHLLSVLCCGLFGLDTLYHDQARKYRFVMHLVDLYGPFALFKGCFDDVNLNKLRLAMTSNHGSLF
NFDPKTIDWDDYFYSVHIPGVLKHMLN

15

SEQUENCE LISTING FREE TEXT:

	SEQ ID NO: 10	OL2707 primer
	SEQ ID NO: 11	OL2708 primer
5	SEQ ID NO: 12	OL2881 primer
	SEQ ID NO: 13	OL2885 primer
	SEQ ID NO: 14	OL2884 primer
	SEQ ID NO: 15	OL2883 primer
	SEQ ID NO: 16	OL2880 primer

10

International Depositary Authority of Canada
Bureau of Microbiology, Health Canada
1015 Arlington Street
Winnipeg, Manitoba, Canada R3E 3R2

Tel: (204) 789-2002
Fax: (204) 789-2036

International Form IDAC/BP/4

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
(issued pursuant to Rule 7.1 of the *Budapest Treaty Regulations*)

ATTACH COPIES OF THE ORIGINAL DEPOSIT CONTRACT AND VIABILITY STATEMENT

This International Depositary Authority accepts the deposit of the microorganism specified below, which was received by it on Jun 7, 2001

To (Name of Depositor) Dr. Gopalan Selvaraj

Address NRC/PBI
110 Gymnasium Place, Saskatoon, SK, S7N 0W9

IDENTIFICATION OF DEPOSIT

Reference assigned by depositor pAMW170

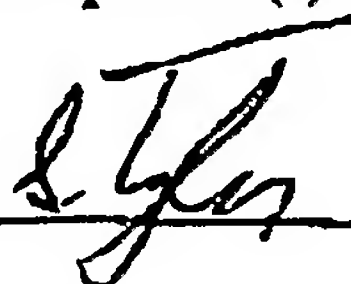
Accession Number assigned by this IDA IDAC 070601-1

The deposit identified above was accompanied by:

☐ a scientific description (specify) _____

☐ a proposed taxonomic designation (specify) _____

Signature of person(s) authorized to represent IDAC:



Date Jun 7, 2001

Receipt in the Case of an Original Deposit

International Depository Authority of Canada
Bureau of Microbiology, Health Canada
1015 Arlington Street
Winnipeg, Manitoba Canada R3E 3R2

Tel: (204) 789-2070
Fax: (204) 789-2097

International Form IDAC/BP/9

STATEMENT OF VIABILITY
(Issued pursuant to Rule 10.2 of the *Budapest Treaty Regulations*)

PARTY TO WHOM THE VIABILITY STATEMENT IS ISSUED

Name Mr. Wayne Anderson

Address National Research Council of Canada
M-58, Montreal Rd., Ottawa, ON, K1A 0R6

DEPOSITOR

Name Dr. Gopalan Selvaraj

Address NRC/PBI
110 Gymnasium Place, Saskatoon, SK, S7N 0W9

IDENTIFICATION OF THE DEPOSIT

Accession Number given by the International Depository Authority IDAC 070601-1

Date of the original deposit (or most recent relevant date) Jun 7, 2001

VIABILITY TEST

The viability of the deposit identified above was tested on (most recent test date) June 8, 2001

On the date indicated above, the culture was:

☒ viable

☐ no longer viable

Conditions under which the Viability Test were performed (to be filled in if the information has been requested and the results of the test were negative) _____

Signature of person(s) authorized to represent IDAC


Date June 8, 2001

Statement of Viability

International Depository Authority of Canada
Bureau of Microbiology, Health Canada
1015 Arlington Street
Winnipeg, Manitoba, Canada R3E 3R2

Tel: (204) 789-2002
Fax: (204) 789-2036

International Form IDAC/BP/4

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
(issued pursuant to Rule 7.1 of the *Budapest Treaty Regulations*)

ATTACH COPIES OF THE ORIGINAL DEPOSIT CONTRACT AND VIABILITY STATEMENT

This International Depository Authority accepts the deposit of the microorganism specified below, which was received by it on Jun 7, 2001

To (Name of Depositor) Dr. Gopalan Selvaraj

Address NRC/PBI
110 Gymnasium Place, Saskatoon, SK, S7N 0W9

IDENTIFICATION OF DEPOSIT

Reference assigned by depositor pAMW133

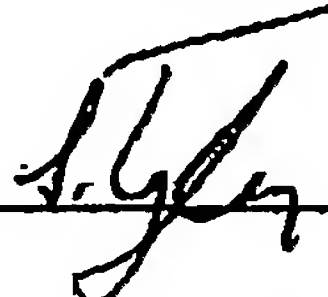
Accession Number assigned by this IDA IDAC 070601-2

The deposit identified above was accompanied by:

☐ a scientific description (specify) _____

☐ a proposed taxonomic designation (specify) _____

Signature of person(s) authorized to represent IDAC:



Date Jun 7, 2001

Receipt in the Case of an Original Deposit

International Depository Authority of Canada
Bureau of Microbiology, Health Canada
1015 Arlington Street
Winnipeg, Manitoba Canada R3E 3R2

Tel: (204) 789-2070
Fax: (204) 789-2097

International Form IDAC/BP/9

STATEMENT OF VIABILITY
(Issued pursuant to Rule 10.2 of the *Budapest Treaty Regulations*)

PARTY TO WHOM THE VIABILITY STATEMENT IS ISSUED

Name Mr. Wayne Anderson
Address National Research Council of Canada
M-58, Montreal Rd., Ottawa, ON, K1A 0R6

DEPOSITOR

Name Dr. Gopalan Selvaraj
Address NRC/PBI
110 Gymnasium Place, Saskatoon, SK, S7N 0W9

IDENTIFICATION OF THE DEPOSIT

Accession Number given by the International Depository Authority IDAC 070601-2
Date of the original deposit (or most recent relevant date) Jun 7, 2001

VIABILITY TEST

The viability of the deposit identified above was tested on (most recent test date) June 8, 2001

On the date indicated above, the culture was:

- ☒ viable
☐ no longer viable

Conditions under which the Viability Test were performed (to be filled in if the information has been requested and the results of the test were negative) _____

Signature of person(s) authorized to represent IDAC


Date June 8, 2001

Statement of Viability

References

- Aarts MGM, Dirkse WG, Stiekma WJ, Pereira A** (1993) Transposon tagging of a male sterility gene in *Arabidopsis*. *Nature* **353**: 715-717
- Aarts MGM, Hodge R, Kalantidis K, Florack D, Wilson ZA, Mulligan B, Stiekma WJ, Scott R, Pereira A** (1997) The *Arabidopsis* *MALE STERILITY 2* protein shares similarity with reductases in elongation condensation complexes. *Plant Journal* **12**: 615-623
- Aarts MGM, Keijzer CJ, Stiekma WJ, Pereira A** (1995) Molecular characterization of the CER1 gene of *Arabidopsis* involved in epicuticular wax biosynthesis and pollen fertility. *Plant Cell* **7**: 2115-2127
- Aguirre PJ, Smith AG** (1993) Molecular characterization of a gene encoding a cysteine-rich protein preferentially expressed in anthers of *Lycopersicon esculentum*. *Plant Molecular Biology* **23**: 477-487
- Altschul SF, Madden TL, Schäffer A, Zhang J, Zhang Z, Miller W, Lipman DJ** (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**: 3389-3402
- Bih FY, Wu SSH, Ratnayake C, Walling LL, Nothnagel EA, Huang AHC** (1999) The predominant protein on the surface of maize pollen is an endoxylanase synthesized by a tapetum mRNA with a long 5' leader. *The Journal of Biological Chemistry* **274**: 22884-22894
- Brown SM, Crouch ML** (1990) Characterization of a gene family abundantly expressed in *Oenothera organensis* pollen that shows sequence similarity to polygalacturonase. *Plant Cell* **2**: 263-274
- Chen L, Zhang S, Beachy RN, Fauquet CM** (1998) A protocol for consistent, large-scale production of fertile transgenic rice plants. *Plant Cell Report* **18**: 25-31. *Plant Cell Report* **18**: 25-31
- Cho H-T, Kende H** (1998) Tissue localization of expression in deep water rice. *Plant Journal* **15**: 805-812

- Datla RSS, Hammerlindl JK, Panchuk B, Pelcher LE, Keller W** (1992) Modified binary plant transformation vectors with the wild-type gene encoding NPTII. *Gene* **122**: 383-384
- Fuerstenberg S, Bucciaglia PA, Smith AG** (2000) Molecular characterization of an anther-specific gene from tobacco shows sequence similarity to a tapetum-specific gene from tomato. *Sex Plant Reprod* **12**: 250-252
- Futamura N, Mori H, Kouchi H, Shinohara K** (2000) Male flower-specific expression of gene for polygalacturonase, pectin methylesterase and β -1,3-glucanase in a dioecious willow (*Salix gilgiana* Seemen). *Plant Cell Physiology* **41**: 16-26
- Goldberg RB, Beals TP, Sanders PM** (1993) Anther development: basic principles and practical applications. *Plant Cell* **5**: 1217-1229
- Hernández-Pinzón I, Ross JHE, Barnes KA, Damant AP, Murphy DJ** (1999) Composition and role of tapetal lipid bodies in the biogenesis of the pollen coat of *Brassica napus*. *Planta* **208**: 588-598
- Hird DL, Worral D, Hodge R, Smartt S, Wyatt P, Scott R** (1993) The anther-specific protein encoded by the *Brassica napus* and *Arabidopsis thaliana* a6 gene displays similarity to β -13-galacturonase. *Plant Journal* **4**: 1023-1033
- Hodge R, Paul W, Draper J, Scott R** (1992) Cold-plaque screening: a simple technique for the isolation of low abundance, differentially expressed transcripts from conventional cDNA libraries. *Plant Journal* **2**: 257-267
- Huang J, Hirji R, Adam L, Rozwadowski KL, Hammerlindl JK, Keller WA, Selvaraj G** (2000) Genetic engineering of glycinebetaine production toward enhancing stress tolerance in plants: metabolic limitations. *Plant Physiology* **122**: 747-756
- Jeon JS, Chung YY, Lee S, Yi GH, Oh BH, An G** (1999) Isolation and characterization of an anther-specific gene, RA8, from rice (*Oryza sativa* L.). *Plant Molecular Biology* **39**: 35-44

- Kandasamy MK, McKinney EC, Meagher RB (1999) The late pollen-specific actins in angiosperms. *The Plant Journal* **18**: 681-691
- Katavic, V., Reed, D. W., Taylor, D. C., Giblin, E. M., Barton, D. L., Zou, J.-T., MacKenzie, S. L., Covello, P. S. & Kunst, L. (1995). Alternation of seed fatty acid composition by an ethyl methanesulfonate-induced mutation in *Arabidopsis thaliana* affecting diacylglycerol acyltransferase activity. *Plant Physiology* **108**, 399-409.
- Kolattukudy PE (1970) Reduction of fatty acids to alcohols by cell-free preparations of *Euglena gracilis*. *Biochemistry* **9**: 1095-1102
- 10 Koltunow AM, Truettner J, Cox KH, Wallroth M, Goldberg RB (1990) Different temporal and spatial gene expression patterns occur during anther development. *Plant Cell* **2**: 1201-1224
- Lopez I, Anthony RG, Maciver SK, Jiang C-J, Khan S, Weeds AG, Hussey PJ (1996) Pollen specific expression of maize genes encoding actin deploymerizing factor-like proteins. *Proc Natl Acad Sci USA* **93**: 7415-7420
- 15 Mariani C, de Beuckeleer M, Truettner J, Leemans J, Goldberg RB (1990) Induction of male sterility in plants by a chimaeric ribonuclease gene. *Nature* **347**: 737-741
- 20 Mascarenhas JP (1975) The biochemistry of angiosperm pollen development. *Bot Rev* **41**: 259-314
- May CE, Appels R (1987) Wheat worldwide in "Wheat and Wheat Improvement" (Ed. E.G. Heyne) pp 165-198. Agronomy No. 13. American Society of Agronomy, Inc Madison, Wisconsin, USA
- 25 Metz JG, Pollard MR, Anderson L, Hayes TR, Lassner MW (2000) Purification of a jojoba embryo fatty acyl-coenzyme A reductase and expression of its cDNA in high erucic acid rapeseed. *Plant Physiology* **122**: 635-644
- Millar AA, Clemens S, Zachgo S, Giblin EM, Taylor DC, Kunst L (1999) *CUT1*, an *Arabidopsis* gene required for cuticular wax biosynthesis and
- 30

- pollen fertility, encodes a very-long-chain fatty acid condensing enzyme.
Plant Cell **11**: 825-838
- Nakamura T, Yamamori M, Hirano H, Hidaka S, Nagamine T (1995)**
Production of waxy (amylose-free) wheats. Molecular General Genetics
5 **248**: 253-259
- Nair, R. B., Joy IV, R. W., Kurylo, E., Shi, X., Schnaider, J., Datla, R. S. S.,
Keller, W. A. & Selvaraj, G. (2000).** Identification of a CYP84 family of
cytochrome P450-dependent mono-oxygenase genes in *Brassica napus*
and perturbation of their expression for engineering sinapine reduction in
10 the seeds. *Plant Physiology* **123**, 1623-1634.
- Pearson WR, Lipman DJ (1988)** Improved tools for biological sequence
comparison. Proc Natl Acad Sci USA **85**: 2444-2448
- Pickett AA, Galwey NW (1997)** A further evaluation of hybrid wheat. Plant
Varieties and Seeds **10**: 15-32
- 15 **Piffanelli P, Ross JHE, Murphy DJ (1998)** Biogenesis and function of the
lipidic structures of pollen grains. Sex Plan Reprod **11**: 65-80
- Preuss D, Lemieux B, Yen G, Davis RW (1993)** A conditional sterile mutation
eliminates surface components from *Arabidopsis* pollen and disrupts cell
signaling during fertilization. Genes & Development **7**: 974-985
- 20 **Rafnar T, Griffith IJ, Kuo M, Bond JF, Rogers BL, Klapper DG (1991)**
Cloning of *Amb a 1* (antigen E), the major allergen family of short
ragweed pollen. Journal of Biological Chemistry **266**: 1229-1236
- Rubinelli P, Hu Y, Ma H (1998)** Identification, sequencing analysis and
expression studies of novel anther-specific genes of *Arabidopsis thaliana*.
25 Plant Molecular Biology **37**: 607-619
- Scott RJ** Pollen exine - the sporopollenin enigma and the physics of pattern. In:
Scott RJ, Stead AD (eds) Molecular and cellular aspects of plant
reproduction, pp. 49-81. Cambridge University Press, Cambridge (1994).
- Shivanna KR, Cresti M, Ciampolini F (1997)** Pollen development and pollen-
30 pistil interaction. In: Shivanna KR, Sawhney VK (eds) Pollen

biotechnology for crop production and improvement. Cambridge University Press, Cambridge, United Kingdom.

- 5 **Spencer DF, Schnare MN, Coulthart MB, Gray MW** (1992) Sequence and organization of a 7.2 kb region of wheat mitochondrial DNA containing the large subunit (26S) rRNA gene. *Plant Molecular Biology* **20**: 347-352
- Ting JTL, Wu SSH, Ratnayake C, Huang AHC** (1998) Constituents of the tapetosomes and elaioplasts in *Brassica campestris* tapetum and their degradation and retention during microsporogenesis. *Plant Journal* **16**: 541-551
- 10 **Vioque J, Kolattukudy PE** (1997) Resolution and purification of an aldehyde-generating and an alcohol-generating fatty acyl-CoA reductase from pea leaves (*Pisum sativum* L.). *Arch Biochem Biophys* **340**: 64-72
- Walden AR, Walter C, Gardner RC** (1999) Genes expressed in *Pinus radiata* male cones include homologs to anther-specific and pathogenesis response genes. *Plant Physiology* **121**: 1103-1116
- 15 **Wang A, Carrier K, Chisholm J, Wieczorek A, Huguenot C, Sanfacon H** (1999) Proteolytic processing of tomato ringspot nepovirus 3C-like protease precursors: definition of the domains for the VPg, protease and putative RNA-dependent RNA polymerase. *J Gen Virol* **80**: 799-809
- 20 **Wang A, Fan H, Singsit C, Ozias-Akins P** (1998) Transformation of peanut with a soybean *vspB* promoter-*uidA* chimeric gene. I. Optimization of a transformation system and analysis of GUS expression in primary transgenic tissues and plants. *Physiologia Plantarum* **102**: 38-48
- 25 **Zou J-T, Zhan X-Y, Wu H-M, Wang H, Cheung AY** (1994) Characterization of a rice pollen-specific gene and its expression. *American Journal of Botany* **81**: 552-561

Claims:

1. An isolated and purified nucleotide sequence, characterized in that the nucleotide sequence is endogenously expressed in wheat anthers, and encodes a peptide having fatty acyl Co-A reductase (FAR) activity.
5
2. An isolated and purified nucleotide sequence, characterized in that the nucleotide sequence is selected from:
 - 10 (a) a *TAA1* gene, or a part thereof, or a complement thereof; and
 - (b) a nucleotide sequence encoding a peptide having at least 50% identity to a peptide encoded by a *TAA1* gene, or a part thereof, or a complement thereof;
- 15 said nucleotide sequence encoding a protein or a part thereof, that alters lipid metabolism in a transgenic plant exogenously expressing said nucleotide sequence.
3. An isolated and purified nucleotide sequence according to claim 2,
20 characterized in that the nucleotide sequence is selected from:
 - (a) SEQ ID NO: 1, 3, or 5, or a part thereof, or a complement thereof; and
 - (b) a nucleotide sequence encoding a peptide having at least 50%
25 identity to a peptide encoded by a SEQ ID NO: 1, 3, or 5, or a part thereof, or a complement thereof;

said nucleotide sequence encoding a protein or a part thereof, that alters lipid metabolism in a transgenic plant exogenously expressing said nucleotide sequence.

- 5 4. An isolated and purified nucleotide sequence according to claim 2, characterized in that the nucleotide sequence is selected from:

(a) SEQ ID NO:1, 3, or 5, or a part thereof, or a complement thereof;
and

- 10 (b) a nucleotide sequence encoding a peptide having at least 70% identity to a peptide encoded by a SEQ ID NO: 1, 3, or 5, or a part thereof, or a complement thereof;

15 said nucleotide sequence encoding a protein or a part thereof, that alters lipid metabolism in a transgenic plant exogenously expressing said nucleotide sequence.

- 20 5. An isolated and purified nucleotide sequence according to claim 2, characterized in that the nucleotide sequence is selected from:

(a) SEQ ID NO:1, 3, or 5, or a part thereof, or a complement thereof;
and

- 25 (b) a nucleotide sequence encoding a peptide having at least 90% identity to a peptide encoded by a SEQ ID NO: 1, 3, or 5, or a part thereof, or a complement thereof;

said nucleotide sequence encoding a protein or a part thereof, that alters lipid metabolism in a transgenic plant exogenously expressing said nucleotide sequence.

- 5 6. An isolated and purified nucleotide sequence according to claim 2, characterized in that the nucleotide sequence is selected from:

(a) SEQ ID NO:1, 3, or 5, or a part thereof, or a complement thereof;
and

- 10 (b) a nucleotide sequence encoding a peptide having at least 95% identity to a peptide encoded by a SEQ ID NO: 1, 3, or 5, or a part thereof, or a complement thereof;

15 said nucleotide sequence encoding a protein or a part thereof, that alters lipid metabolism in a transgenic plant exogenously expressing said nucleotide sequence.

- 20 7. An isolated and purified nucleotide sequence according to claim 2, characterized in that the nucleotide sequence is selected from:

(a) SEQ ID NO:1, 3, or 5, or a part thereof, or a complement thereof;
and

- 25 (b) a nucleotide sequence encoding a peptide having at least 99% identity to a peptide encoded by a SEQ ID NO: 1, 3, or 5, or a part thereof, or a complement thereof;

said nucleotide sequence encoding a protein or a part thereof, that alters lipid metabolism in a transgenic plant exogenously expressing said nucleotide sequence.

5 8. An isolated and purified nucleotide sequence according to claim 3, characterized in that the nucleotide sequence is selected from:

 (a) SEQ ID NO: 1, 3, or 5, or a part thereof, or a complement thereof;
and

10 (b) a nucleotide sequence that can hybridize to SEQ ID NO: 1, 3, or 5 under stringent hybridization conditions.

9. An expression cassette, characterized in that the expression cassette comprises the nucleotide sequence according to claim 2, operably linked to a
15 promoter.

10. The expression cassette according to claim 9, characterized in that the nucleotide sequence is oriented in an antisense direction, or in both a sense and an antisense direction to produce double stranded RNA, relative relative to said
20 promoter.

11. A construct, characterized in that the construct comprises the nucleotide sequence according to claim 2.

25 12. The construct according to claim 11, characterized in that the construct includes a promoter, said nucleotide sequence operatively linked to said promoter.

13. A transgenic plant, characterized in that the transgenic plant comprises the construct according to claim 12, said nucleotide sequence expressed from said promoter thereby altering a lipid metabolism of said transgenic plant.
- 5 14. The transgenic plant according to claim 13, characterized in that said plant comprises an increased concentration of fatty alcohols per weight of plant material relative to an unmodified plant.
- 10 15. The transgenic plant according to claim 13, characterized in that said plant comprises a decreased concentration of fatty alcohols per weight of plant material relative to an unmodified plant.
- 15 16. The transgenic plant according to claim 13, characterized in that the transgenic plant comprises pollen grains or seeds having an altered fatty alcohol content relative to pollen grains or seeds of an unmodified plant.
17. The transgenic plant according to claim 13, characterized in that said plant is a species of a woody plant, a non-woody plant, or a grass.
- 20 18. The transgenic plant according to claim 13, characterized in that said plant is selected from the group consisting of crucifer crops, tobacco, wheat, corn, sugar cane, apple, tomato, and berries.
- 25 19. The transgenic plant according to claim 13, characterized in that said construct comprises an organ-specific promoter to direct organ-specific expression of said nucleotide sequence in said transgenic plant.

20. The transgenic plant according to claim 13, characterized in that said transgenic plant exhibits modified characteristics compared to an unmodified plant, said modified characteristics selected from the group consisting of:

- 5 increased pest resistance;
- male sterility;
- reduced height;
- reduced internode spacing;
- increased resistance to wind damage;
- 10 reduced growth rate;
- altered cross-pollination specification;
- increased fruit or nut aesthetic appeal;
- increased fruit or nut shelf-life;
- delayed vegetative development; and
- 15 delayed propagative development.

21. The transgenic plant according to claim 13, characterized in that said nucleotide sequence is oriented for antisense expression from said construct, said transgenic plant exhibiting reduced levels of fatty acyl Co-A reductase compared
20 to an unmodified plant.

22. A plant extract, characterized in that said plant extract is derived from a transgenic plant according to any one of claims 13 to 21 for use in the production of a nutritional, cosmetic or pharmaceutical agent.

25

23. An isolated and purified nucleotide sequence, characterized in that the nucleotide sequence is selected from:

- (a) SEQ ID NO: 7, 8, or 9 or a complement thereof; and

(b) a nucleotide sequence that can hybridize to SEQ ID NO: 7, 8, or 9 or a complement thereof under stringent hybridization conditions.

24. The isolated and purified nucleotide sequence according to claim 23,
5 characterized in that said nucleotide sequence is for use as a hybridization probe, PCR primer or DNA sequencing primer.

25. A promoter sequence, characterized in that said promoter sequence regulates gene expression of anthers and is isolated from a genomic DNA library
10 by chromosome walking, using as a probe the isolated and purified nucleotide sequence according to claim 24.

26. A promoter sequence, characterized in that said promoter sequence comprises a region of about 1.6kb upstream from a start codon of SEQ ID NO: 8.
15

27. A construct, characterized in that said construct comprises a promoter sequence according to any one of claims 23 to 26, operably linked to a nucleotide sequence having an open reading frame, or a part thereof, or a complement thereof.
20

28. A transgenic plant, characterized in that said transgenic plant is transformed with a construct according to claim 27, said promoter inducing expression of said open-reading frame, or a part thereof, or a complement thereof, in at least one anther of said transgenic plant.
25

29. The transgenic plant according to claim 28, characterized in that said open-reading frame encodes an anther or pollen inactivating gene, and expression of said open-reading frame induces male sterility of said transgenic plant.

30. The transgenic plant according to claim 28, characterized in that said open-reading frame encodes a transposase, and expression of said open-reading frame induces an increased rate of genomic DNA rearrangement in anther or pollen cells of said transgenic plant.

5

31. The transgenic plant according to claim 28, characterized in that said open-reading frame encodes a peptide suitable for use as a nutritional or pharmaceutical agent, said peptide expressed in anthers or pollen of said transgenic plant.

10

32. The transgenic plant according to claim 28, characterized in that said open-reading frame encodes a peptide required for the production of a nutritional or pharmaceutical agent.

15

33. The transgenic plant according to claim 28, characterized in that said open-reading frame encodes a protein that inhibits the production and / or accumulation of an unwanted substance selected from the group consisting of a toxin, and an allergen.

20

34. The transgenic plant according to claim 28, characterized in that said open-reading frame encodes a peptide for altering a cross-pollination specification of said transgenic plant.

25

35. The transgenic plant according to claim 28, characterized in that said open reading frame is oriented for antisense expression within said construct, thereby inducing antisense repression of an endogenous gene expression within anthers or pollen of said transgenic plant.

30

36. The transgenic plant according to claim 32, characterized in that said nutritional agent is Octacosanol.

37. A plant extract, characterized in that the plant extract is derived from a transgenic plant according to any one of claims 28 to 36.

5 38. The transgenic plant according to claim 13, characterized in that the plant comprises fruit, and the plant exhibits increased levels of fatty alcohols in said fruit to preserve and / or provide an enhanced appearance of said fruit.

39. Use for Octacosanol derived from the transgenic plant according to claim
10 36, characterized in that said Octacosanol is for use as a nutritional supplement.

40. Use for fatty alcohols derived from the transgenic plant according to claim 13, characterized in that said use is selected from:

15 use as a wax;
use as a cleaning agent;
use as a cosmetic agent;
use as a dermatological agent;
use as a pharmaceutical agent;
20 use as a nutritional agent; and
use as a coating agent.

41. An isolated and purified peptide, characterized in that said isolated and purified peptide is selected from the group consisting of:

25

(a) SEQ ID NO: 2, 4, or 6, or a part thereof; and
(b) a peptide having at least 50% identity to a peptide encoded by a
SEQ ID NO: 2, 4, or 6, or a part thereof;

said peptide or a part thereof altering lipid metabolism in a transgenic plant when generated by exogenous gene expression in said transgenic plant.

42. An isolated and purified peptide according to claim 41, characterized in
5 that said isolated and purified peptide is selected from the group consisting of:

- (a) SEQ ID NO: 2, 4, or 6, or a part thereof; and
- (b) a peptide having at least 70% identity to a peptide encoded by a

SEQ ID NO: 2, 4, or 6, or a part thereof;

10

said peptide or a part thereof altering lipid metabolism in a transgenic plant when generated by exogenous gene expression in said transgenic plant.

43. An isolated and purified peptide according to claim 41, characterized in
15 that said isolated and purified peptide is selected from the group consisting of:

- (a) SEQ ID NO: 2, 4, or 6, or a part thereof; and
- (b) a peptide having at least 90% identity to a peptide encoded by a

SEQ ID NO: 2, 4, or 6, or a part thereof;

20

said peptide or a part thereof altering lipid metabolism in a transgenic plant when generated by exogenous gene expression in said transgenic plant.

44. An isolated and purified peptide according to claim 41, characterized in
25 that said isolated and purified peptide is selected from the group consisting of:

- (a) SEQ ID NO: 2, 4, or 6, or a part thereof; and
- (b) a peptide having at least 95% identity to a peptide encoded by a

SEQ ID NO: 2, 4, or 6, or a part thereof;

said peptide or a part thereof altering lipid metabolism in a transgenic plant when generated by exogenous gene expression in said transgenic plant.

- 5 45. An isolated and purified peptide according to claim 41, characterized in that said isolated and purified peptide is selected from the group consisting of:

 (a) SEQ ID NO: 2, 4, or 6, or a part thereof; and

 (b) a peptide having at least 99% identity to a peptide encoded by a

- 10 SEQ ID NO: 2, 4, or 6, or a part thereof;

said peptide or a part thereof altering lipid metabolism in a transgenic plant when generated by exogenous gene expression in said transgenic plant.

- 15 46. A pharmaceutical composition, characterized in that the pharmaceutical composition comprises a fatty alcohol derived from the transgenic plant according to any one of claims 13 to 21 and 28 to 37, together with an excipient or carrier.

- 20 47. A pharmaceutical composition, characterized in that the pharmaceutical composition comprises the plant extract according to claim 22 or claim 37, together with an excipient or carrier.

48. A nutritional composition, characterized in that the nutritional
25 composition comprises a fatty alcohol derived from the transgenic plant according to any one of claims 13 to 21 and 28 to 37 together with an excipient or carrier.

49. A nutritional composition, characterized in that the nutritional composition comprises the plant extract according to claim 22 or claim 37 together with an excipient or carrier.
- 5 50. A method of treating or preventing a medical condition, characterized in that the method comprises administration a pharmaceutical composition according to claim 46 or claim 47.
- 10 51. A method of providing a dietary supplement, characterized in that the method comprises administration of a nutritional composition according to claim 48 or claim 49.
- 15 52. A method for the production and isolation of fatty alcohols, characterized in that the method comprises the steps of:
transforming an organism with a construct according to claim 11;
growing or propagating said organism containing said construct; and
extracting said fatty alcohols from said organism.
- 20 53. A method according to claim 52, characterized in that said organism is an *E.coli* bacterium.
54. A method according to claim 52, characterized in that said organism is a plant, or a plant embryo.
- 25 55. A method according to claim 52, characterized in that said organism is a tobacco plant, or a tobacco plant embryo.
- 30 56. Isolated and purified fatty alcohols, characterized in that said fatty alcohols are obtained by a method according to any one of claims 52 to 54.

57. A method of inducing dwarfism in a plant, characterized in that the method comprises the steps of:
transforming a plant cell, plant embryo or plant with a construct according
5 to claim 11;
growing or propagating said plant cell, plant embryo, or plant, thereby
generating a plant expressing a DNA sequence encoded by said construct,
said plant having a reduced size compared to an unmodified plant.
- 10 58. A plant characterized in that the plant is generated by the method according to claim 57.
59. The plant according to claim 58, characterized in that said plant exhibits an increased resistance to wind damage compared to an unmodified plant.

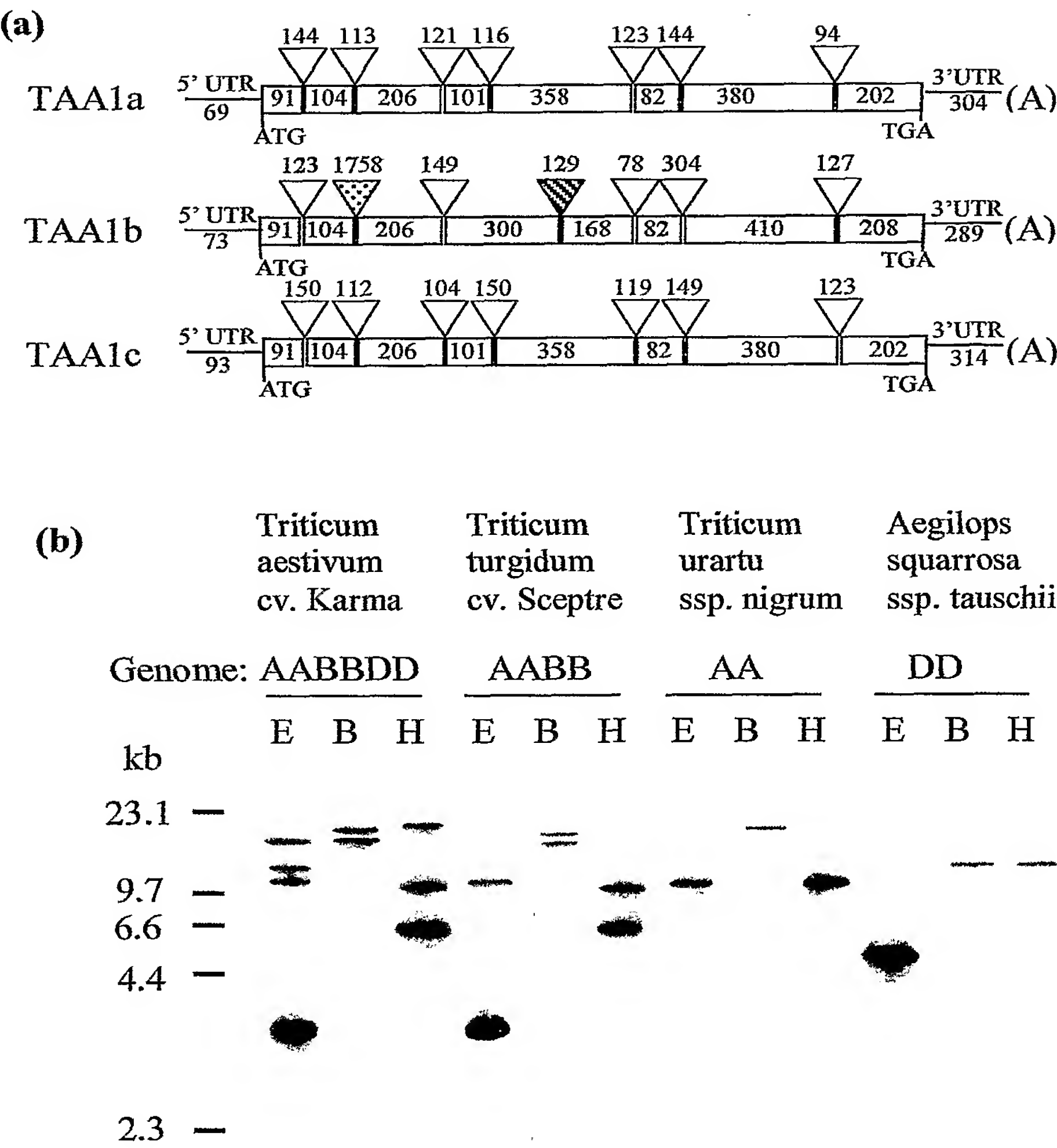


FIG. 1

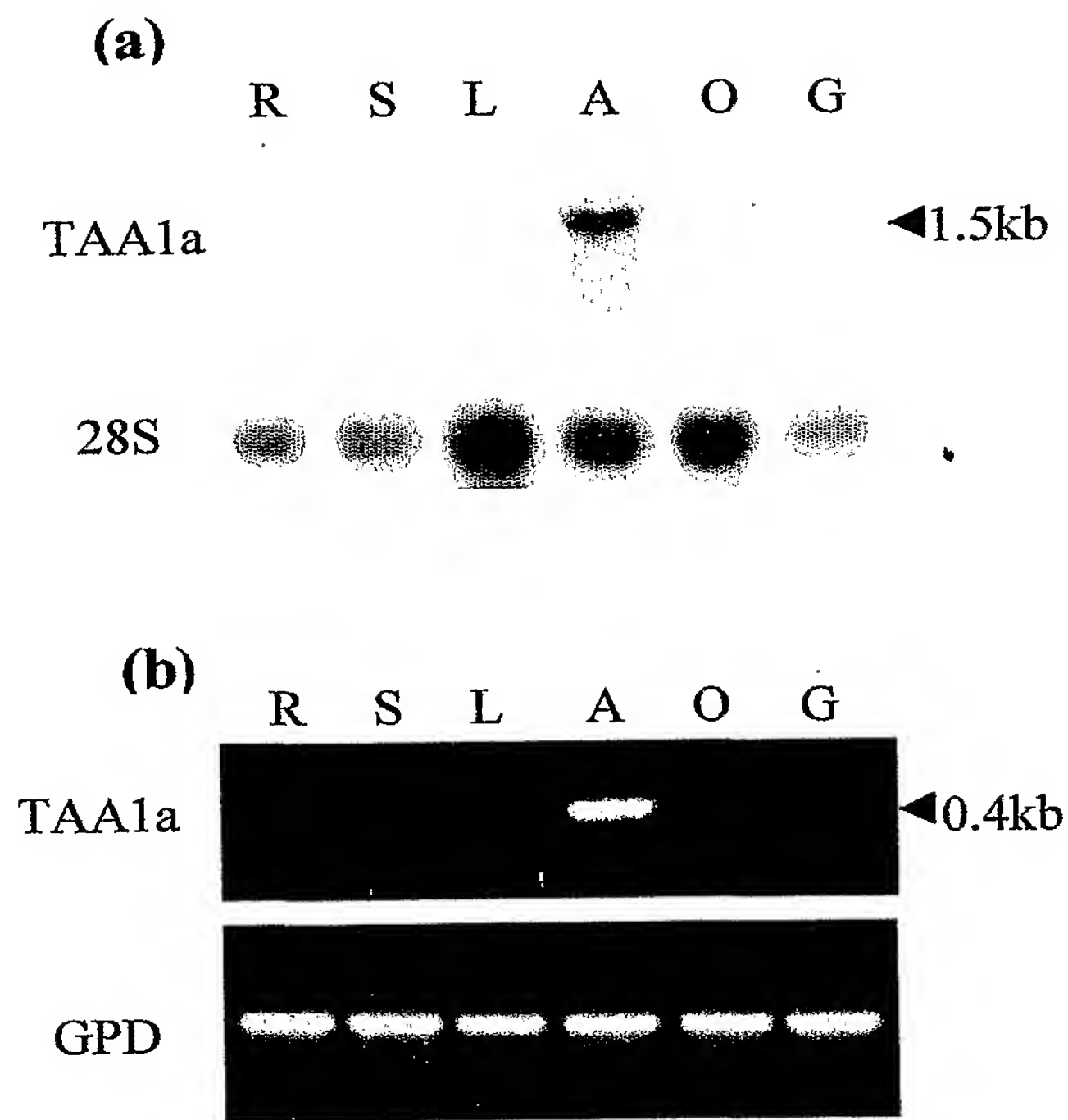
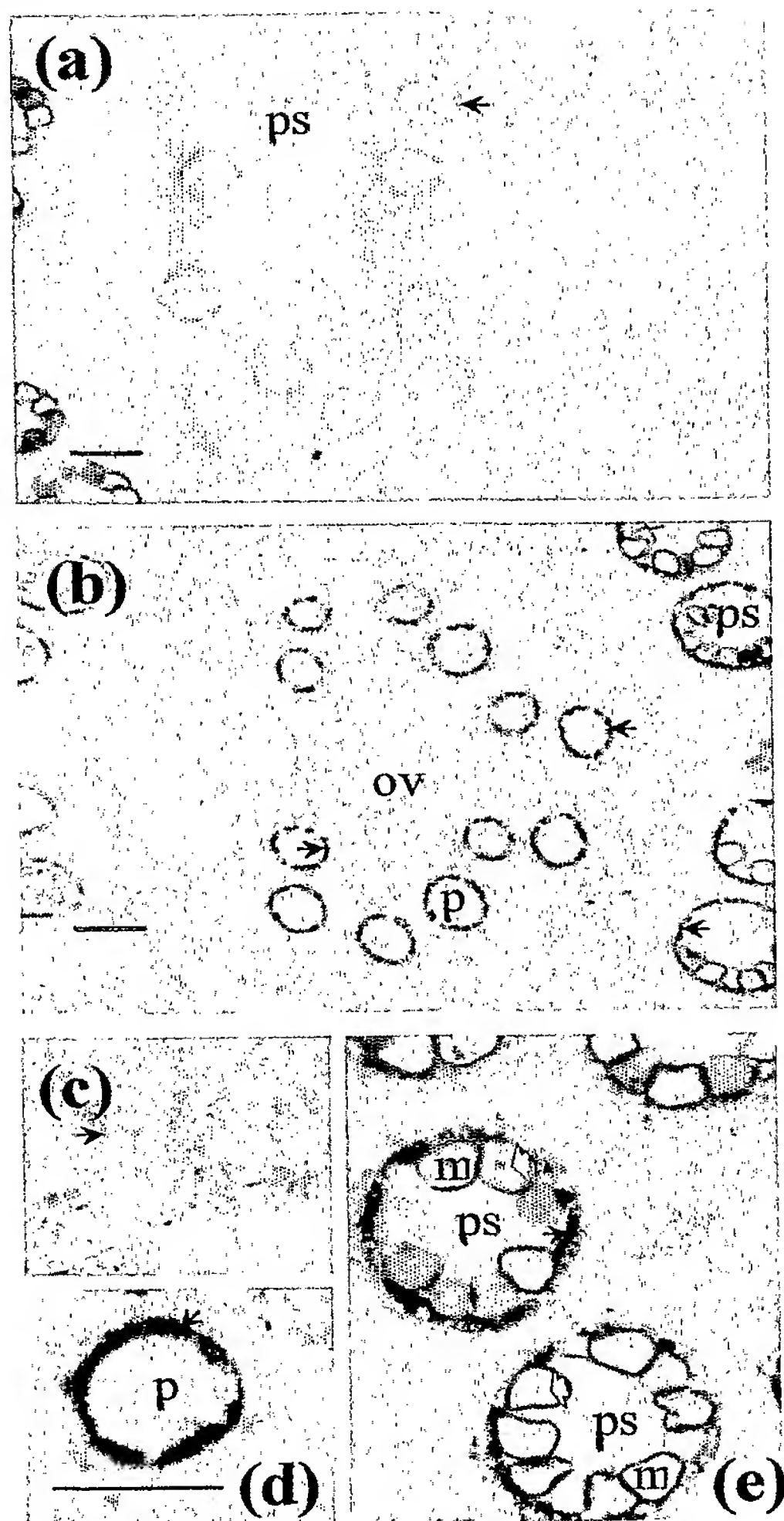
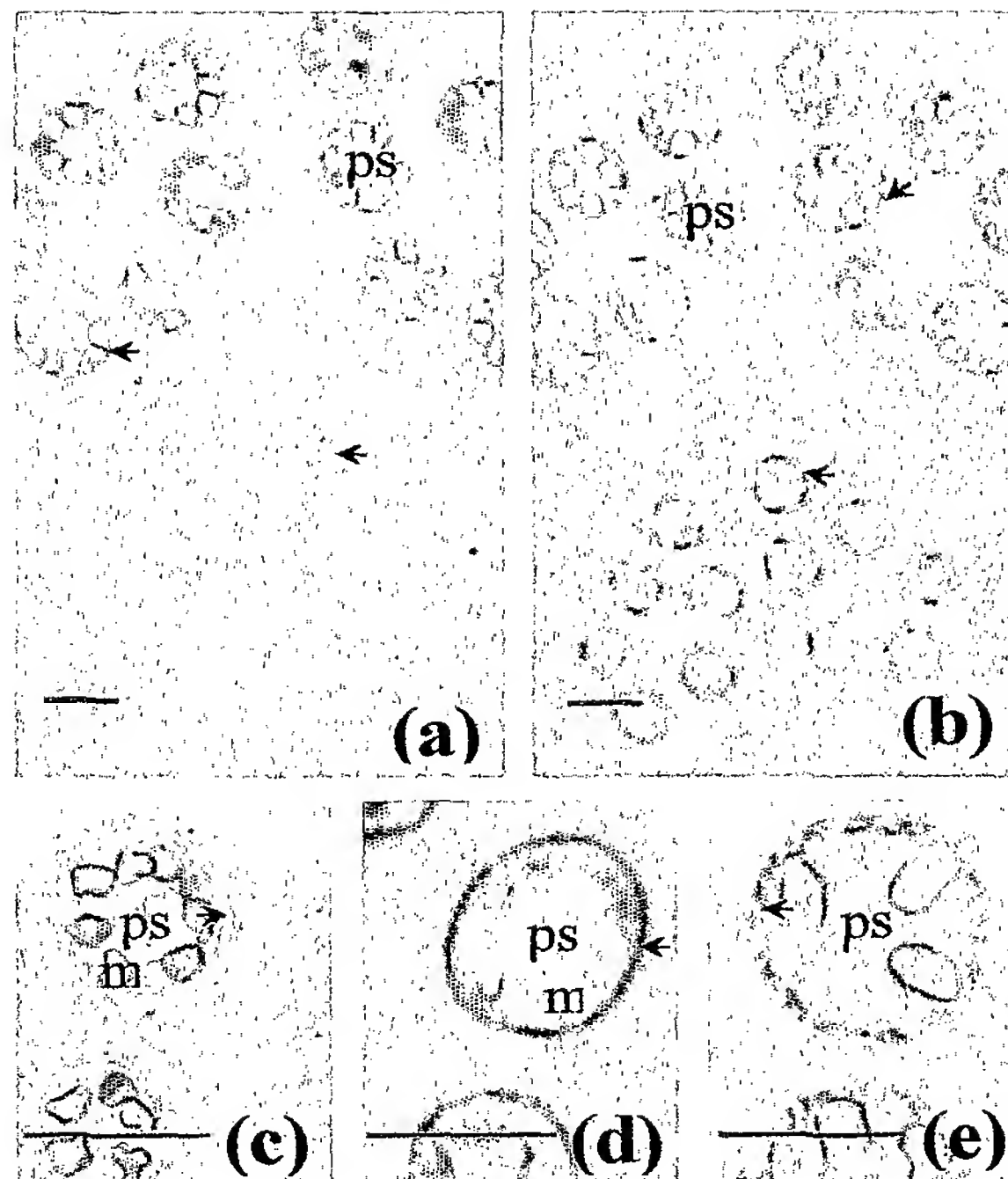


FIG. 2

3 / 9

**FIG. 3**

4 / 9

**FIG. 4**

5 / 9

(a)

TAA1a 9 NIIGYFKNKSILITGSTGFLGKILVEKILRVQPDVKKIYLPVRAVDAAAAKHRVETE VVG 68
 FAR 6 S-LEFLD--A--V--A--S-A--F---V--S--N---L--LL--T-DET-AL-LQN--F- 65

TAA1a 69 KELFGLLREKHGGRFQSFIWEKIVPLAGDVMREDFGVDSETLREL RVTQE L DVIVNGAAT 128
 FAR 66 ----KV-KQNL-AN-Y--VS--VTVVP--ITG--LCLKDVN-K-ĆEMWR-I--V--L--- 124

TAA1a 129 TNFYERYDVALDVNVMGVKHCNFAKKCPNLKVLLHVSTAYVAGEKQGLVQERP FKNGET 188
 FAR 125 I--I-----S-LI-TY-A-YVLD-----NK--IFV-----S---N--IL-K-YYM--S 184

TAA1a 189 LLEGTRLDDIDTELKLA KDLKKQLEADV DSSPKAERKAMKDLGLTRARHFRWPNTYVFTKS 248
 FAR 185 -NGRLG---NV-K--VEAKINE-Q-ĆAGATE-SIKST---M-IE----WG--VY----A 243

TAA1a 249 MGEMVLSQLQCDVPVVIVRPSIITSVQNDPLPGWIEGTRTIDTIVIGYAKQNLTYFLADL 308
 FAR 244 L---L-M-YKG-IPLT-I--T---TFKE-F---V--V----NVPVY-G-GR-RCM-CGP 303

TAA1a 309 NLTMDVMPGDMVNVNAMMAAIVAHSSSSLEKTKSHPKQHAPAVYHVSSSLRNPAPYNVLHE 368
 FAR 304 STII-LI-A-----TIV-M---ANQĆĆĆĆĆĆCRYVE-VT---G--AA--MKLSA-P- 354

TAA1a 369 AGFRYFTEHPRVGPDGRTVRTHKMTFLSSMASFHLFMMMLRYRL LLELLHLLSILCCGLFG 428
 FAR 355 MAH---TKN-WIN--RNP-HVGRAMVF--FST---YLT-NFL-P-KV-EIANTIF-QWFC 413

TAA1a 429 LDTLYHDQARKYRFVMHLVDLYGPFALFKGCFDDVNLNKLRLAMTSNĆCHGSLENFDPKT 486
 FAR 414 ĆKGK-M-LK--T-LLLR---I-K-YLF-Q-I---M-TE---I-AKESIVEADM-Y---RA 472

TAA1a 487 IDWDEYFYRVHIPGVIKYML 506
 FAR 473 -N-ED--LKT-F---VEHV- 492

(b)

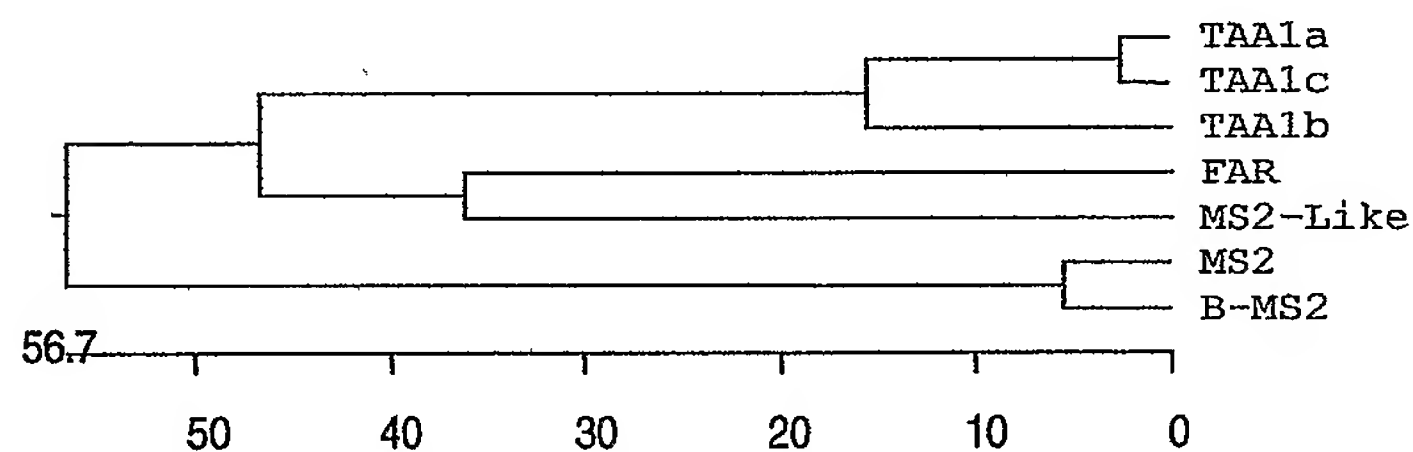


FIG.5

6 / 9

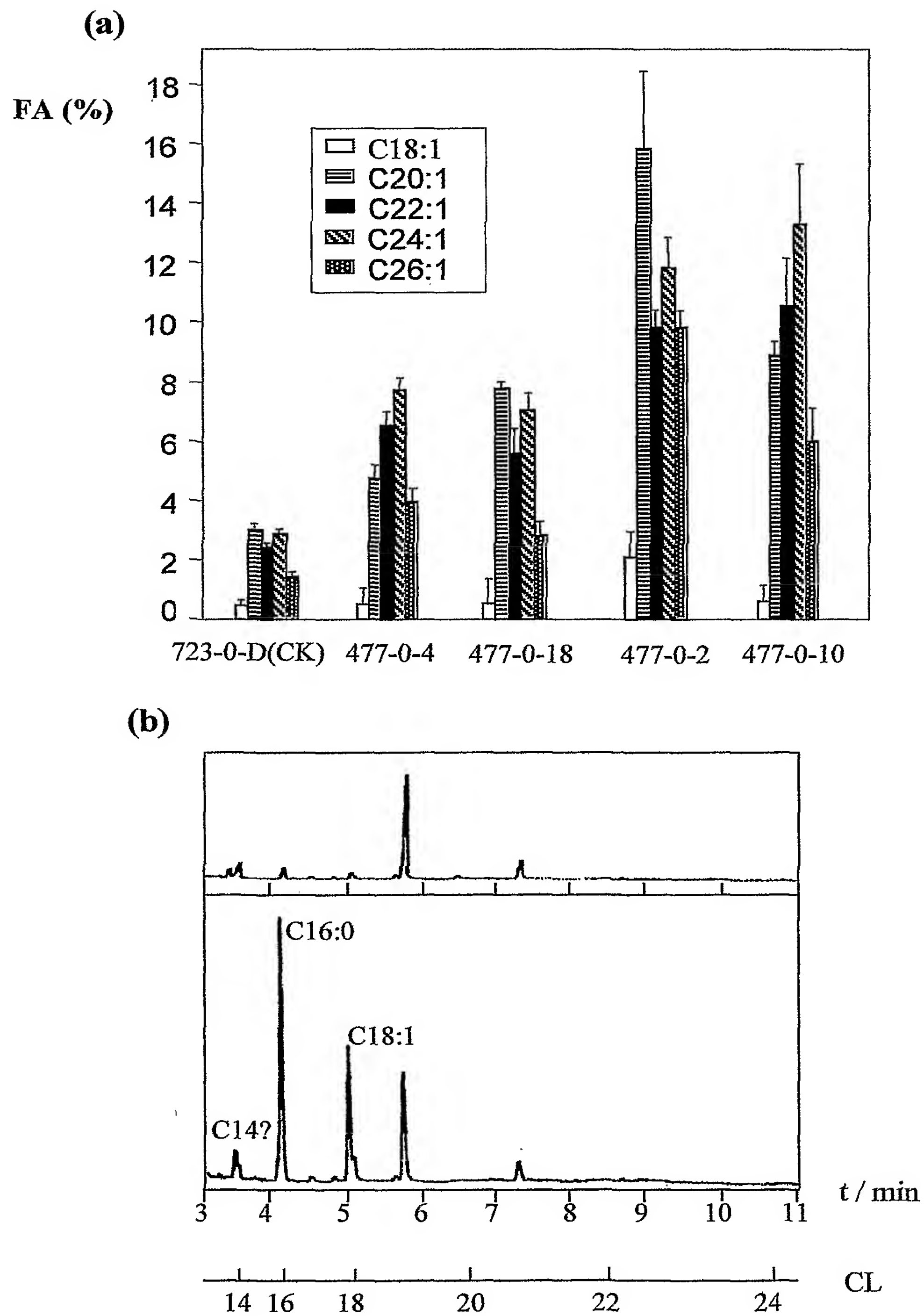
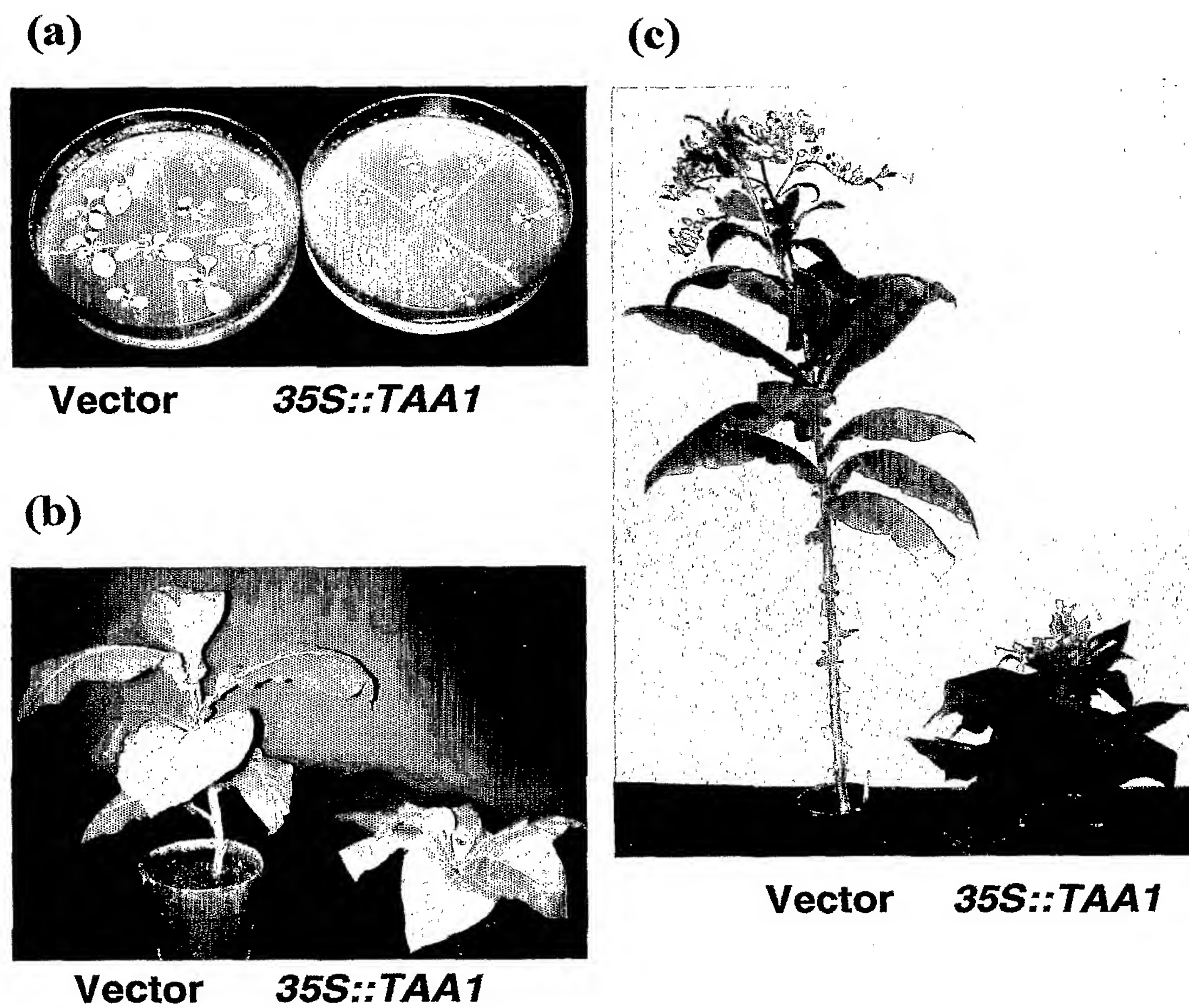


FIG. 6

**FIG. 7**

8 / 9

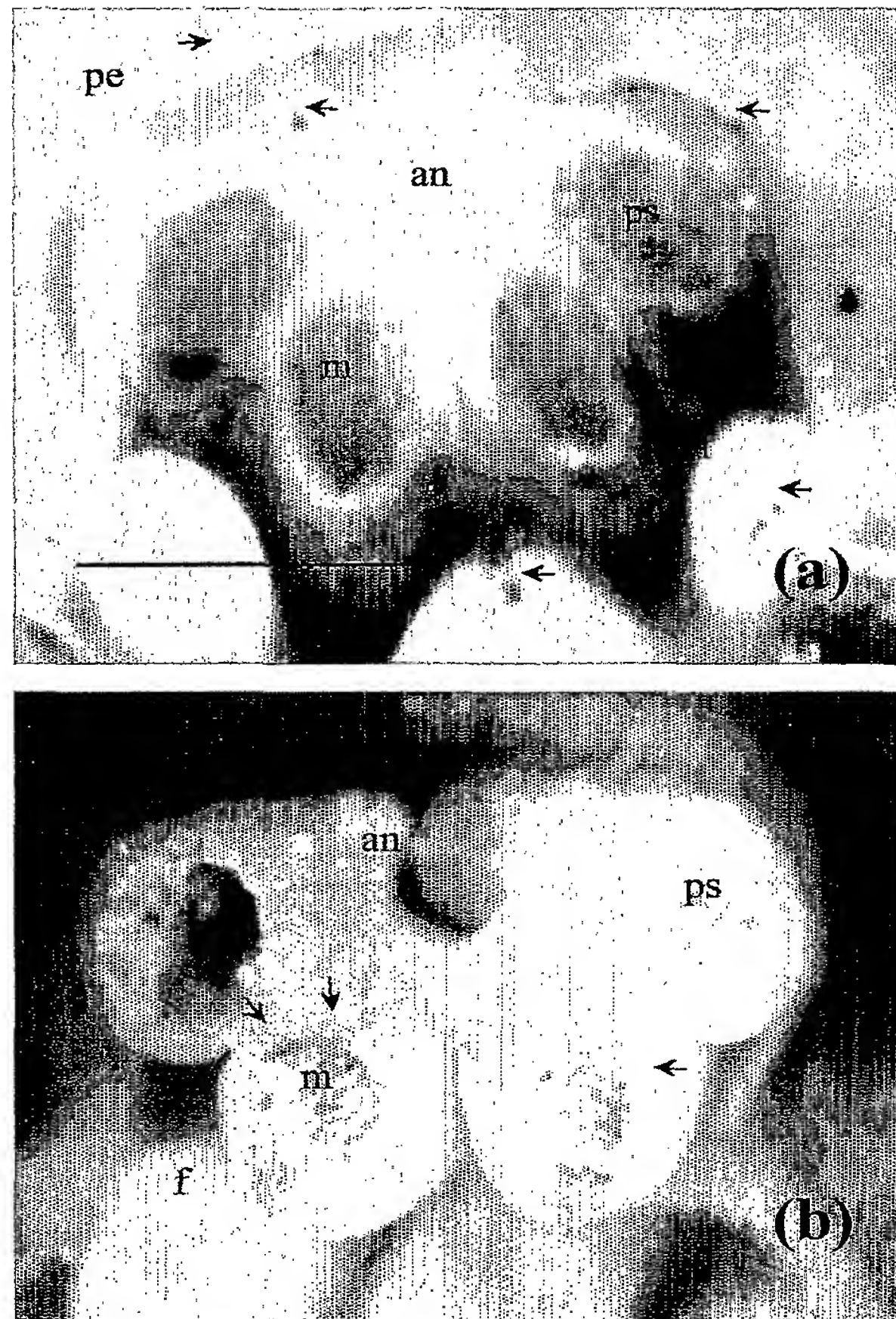
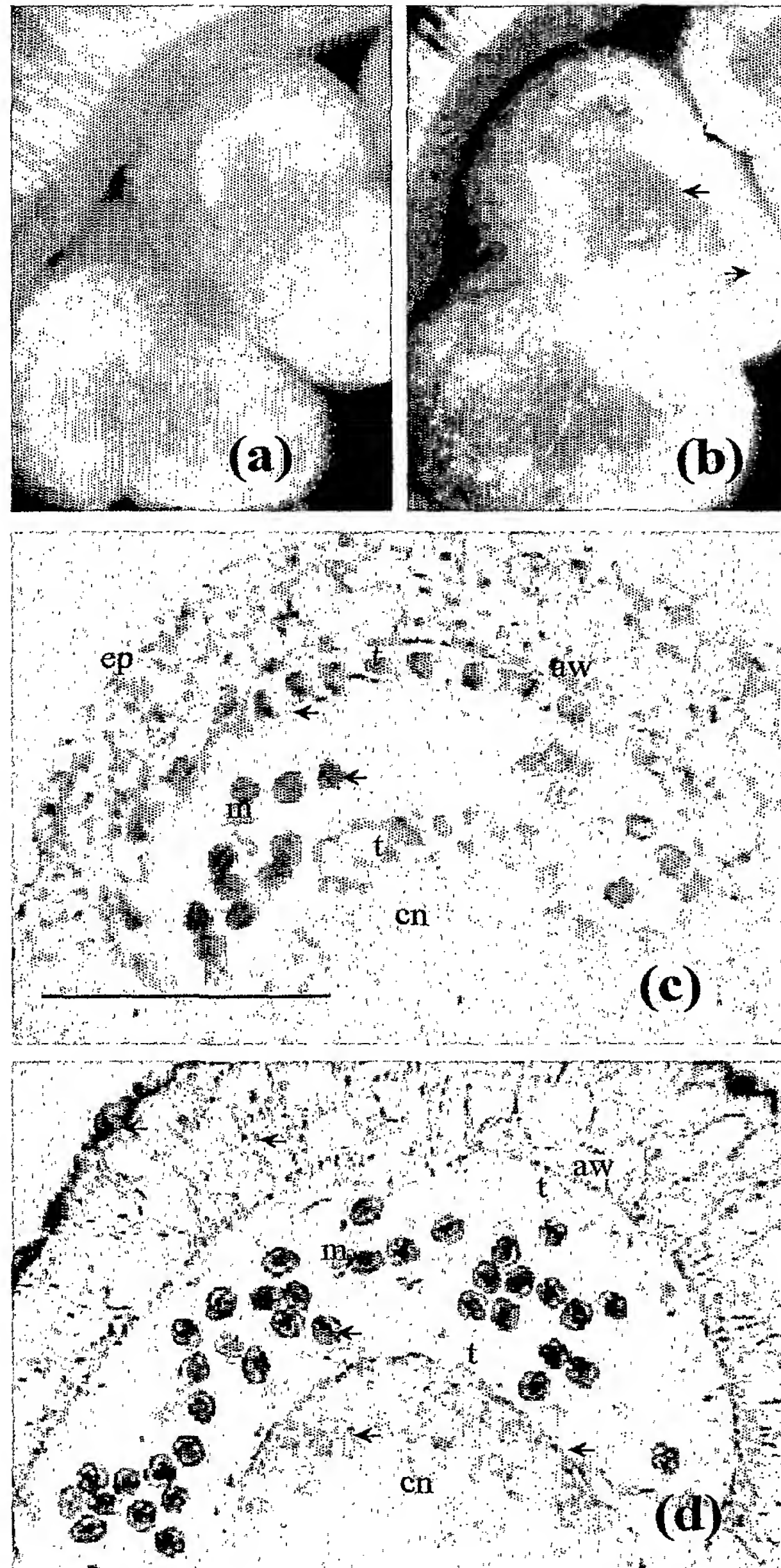


FIG. 8

9 / 9

**FIG. 9**

1 / 27

SEQUENCE LISTING

<110> National Research Council of Canada

<120> Anther-specific TAA1 genes encoding fatty acyl Co-A reductases,
and uses thereof

<130> 46450-PT

<150> US 60/296,159

<151> 2001-06-07

<160> 16

<170> PatentIn version 3.1

<210> 1

<211> 1919

<212> DNA

<213> Triticum aestivum

<220>

<221> CDS

<222> (70) .. (1593)

<223>

<400> 1

ctcctctctc tctctctctc tctctctctc tcgcacgcac gcacgcacgc acacaagaaa 60

aaaatcaag atg gtt gac aca ctg agt gaa gag aac atc att gga tac ttc 111

Met Val Asp Thr Leu Ser Glu Glu Asn Ile Ile Gly Tyr Phe

1

5

10

aag aac aag agc atc ctc atc act gga tca aca ggc ttt ctt gga aag 159

Lys Asn Lys Ser Ile Leu Ile Thr Gly Ser Thr Gly Phe Leu Gly Lys

15

20

25

30

ata ctg gtg gag aag ata ctg aga gtt caa cct gat gtg aag aag atc 207

Ile Leu Val Glu Lys Ile Leu Arg Val Gln Pro Asp Val Lys Lys Ile

35

40

45

tac ctc ccg gtg cga gcg gtg gat gcc gcg gcg gca aag cat cgg gtg 255

Tyr Leu Pro Val Arg Ala Val Asp Ala Ala Ala Ala Lys His Arg Val

50

55

60

gag act gag gtg gta ggg aag gag ttg ttc ggg ctt ctg agg gag aag 303

Glu Thr Glu Val Val Gly Lys Glu Leu Phe Gly Leu Leu Arg Glu Lys

65

70

75

cac ggg ggc agg ttt caa tct ttc atc tgg gaa aag atc gtc cca ttg 351

His Gly Gly Arg Phe Gln Ser Phe Ile Trp Glu Lys Ile Val Pro Leu

80

85

90

2 / 27

gcc	gga	gac	gtg	atg	cgc	gag	gac	ttc	ggc	gtc	gac	agc	gag	acc	ctg	399
Ala	Gly	Asp	Val	Met	Arg	Glu	Asp	Phe	Gly	Val	Asp	Ser	Glu	Thr	Leu	
95					100					105					110	
agg	gag	ctc	cgg	gtg	acc	cag	gag	ctc	gat	gtc	atc	gtt	aat	ggc	gcc	447
Arg	Glu	Leu	Arg	Val	Thr	Gln	Glu	Leu	Asp	Val	Ile	Val	Asn	Gly	Ala	
				115					120					125		
gcc	acc	acc	aac	ttc	tac	gaa	agg	tat	gat	gtg	gct	cta	gac	gtg	aac	495
Ala	Thr	Thr	Asn	Phe	Tyr	Glu	Arg	Tyr	Asp	Val	Ala	Leu	Asp	Val	Asn	
			130					135					140			
gtg	atg	gga	gtg	aag	cat	atg	tgc	aac	ttc	gcc	aag	aag	tgc	ccc	aat	543
Val	Met	Gly	Val	Lys	His	Met	Cys	Asn	Phe	Ala	Lys	Lys	Cys	Pro	Asn	
		145					150						155			
ctc	aag	gtg	ctc	ctc	cat	gtc	tcc	acg	gct	tac	gtg	gcg	ggt	gag	aag	591
Leu	Lys	Val	Leu	Leu	His	Val	Ser	Thr	Ala	Tyr	Val	Ala	Gly	Glu	Lys	
	160					165					170					
caa	ggg	ctg	gtg	caa	gag	aga	cca	ttc	aag	aat	ggc	gag	acg	ctg	ctc	639
Gln	Gly	Leu	Val	Gln	Glu	Arg	Pro	Phe	Lys	Asn	Gly	Glu	Thr	Leu	Leu	
175					180					185					190	
gag	ggg	acc	cgc	ctc	gac	atc	gac	act	gag	ctg	aaa	ctg	gcc	aag	gac	687
Glu	Gly	Thr	Arg	Leu	Asp	Ile	Asp	Thr	Glu	Leu	Lys	Leu	Ala	Lys	Asp	
				195					200					205		
ctg	aaa	aag	cag	ctt	gag	gcc	gac	gtt	gat	tcg	tcg	ccc	aag	gcc	gaa	735
Leu	Lys	Lys	Gln	Leu	Glu	Ala	Asp	Val	Asp	Ser	Ser	Pro	Lys	Ala	Glu	
			210					215					220			
agg	aag	gcc	atg	aag	gat	ctt	ggc	ctt	acc	agg	gcc	cgg	cac	ttc	agg	783
Arg	Lys	Ala	Met	Lys	Asp	Leu	Gly	Leu	Thr	Arg	Ala	Arg	His	Phe	Arg	
		225					230					235				
tgg	cca	aac	aca	tac	gtg	ttc	acc	aag	tcg	atg	ggg	gag	atg	gtg	cta	831
Trp	Pro	Asn	Thr	Tyr	Val	Phe	Thr	Lys	Ser	Met	Gly	Glu	Met	Val	Leu	
	240					245					250					
agc	cag	ttg	cag	tgt	gat	gtc	ccc	gtt	gtc	atc	gtc	cgt	ccc	agc	atc	879
Ser	Gln	Leu	Gln	Cys	Asp	Val	Pro	Val	Val	Ile	Val	Arg	Pro	Ser	Ile	
255					260					265					270	
atc	aca	agt	gtc	cag	aac	gac	cca	ctg	ccc	gga	tgg	atc	gaa	ggc	acc	927
Ile	Thr	Ser	Val	Gln	Asn	Asp	Pro	Leu	Pro	Gly	Trp	Ile	Glu	Gly	Thr	
				275					280					285		
agg	acg	atc	gac	acg	atc	gtg	atc	ggc	tat	gcg	aag	cag	aac	ctg	aca	975
Arg	Thr	Ile	Asp	Thr	Ile	Val	Ile	Gly	Tyr	Ala	Lys	Gln	Asn	Leu	Thr	
			290					295					300			

3 / 27

tac ttc ttg gcc gac ctc aac ctc acc atg gat gtg atg ccg ggc gac	1023
Tyr Phe Leu Ala Asp Leu Asn Leu Thr Met Asp Val Met Pro Gly Asp	
305 310 315	
atg gtg gtg aat gcg atg atg gcg gca ata gtg gca cac agc tcg tcc	1071
Met Val Val Asn Ala Met Met Ala Ala Ile Val Ala His Ser Ser Ser	
320 325 330	
tca ttg gag aag aca aag tca cat ccc aag caa cat gca ccg gcg gtg	1119
Ser Leu Glu Lys Thr Lys Ser His Pro Lys Gln His Ala Pro Ala Val	
335 340 345 350	
tac cac gtg agc tcg tcg ctg cgt aat ccg gca cca tac aat gtg ctt	1167
Tyr His Val Ser Ser Ser Leu Arg Asn Pro Ala Pro Tyr Asn Val Leu	
355 360 365	
cat gag gct ggg ttt cgg tac ttc acg gag cac cct cgc gtg ggc cct	1215
His Glu Ala Gly Phe Arg Tyr Phe Thr Glu His Pro Arg Val Gly Pro	
370 375 380	
gac ggt cgc acc gtg cgt acc cat aag atg aca ttc ctc agc agc atg	1263
Asp Gly Arg Thr Val Arg Thr His Lys Met Thr Phe Leu Ser Ser Met	
385 390 395	
gct tcc ttc cac cta ttt atg atg ctc agg tac cgc ctc ctc ctc gag	1311
Ala Ser Phe His Leu Phe Met Met Leu Arg Tyr Arg Leu Leu Leu Glu	
400 405 410	
ctc ctc cac ctg ctc tcc atc ctc tgc tgc ggc ctc ttc ggc ctc gac	1359
Leu Leu His Leu Leu Ser Ile Leu Cys Cys Gly Leu Phe Gly Leu Asp	
415 420 425 430	
acc ctc tac cac gac caa gca cgc aag tac agg ttc gtg atg cac ctg	1407
Thr Leu Tyr His Asp Gln Ala Arg Lys Tyr Arg Phe Val Met His Leu	
435 440 445	
gtg gat ctg tac ggg ccc ttt gcg ctg ttc aag ggg tgc ttc gat gac	1455
Val Asp Leu Tyr Gly Pro Phe Ala Leu Phe Lys Gly Cys Phe Asp Asp	
450 455 460	
gtc aac cta aac aag ctc agg ctc gcc atg acc agc aac cat ggt agc	1503
Val Asn Leu Asn Lys Leu Arg Leu Ala Met Thr Ser Asn His Gly Ser	
465 470 475	
ctc ttc aat ttc gac ccg aag acc att gat tgg gac gag tac ttc tac	1551
Leu Phe Asn Phe Asp Pro Lys Thr Ile Asp Trp Asp Glu Tyr Phe Tyr	
480 485 490	
agg gtc cac atc ccc ggg gtc ata aag tac atg ctc aag tga	1593
Arg Val His Ile Pro Gly Val Ile Lys Tyr Met Leu Lys	
495 500 505	
aatatccgtg caccgaaatt caggcgttgc cttaataatt aaataatcgt acgattgtaa	1653

4/27

gaaatgtcct cccaaattgg gttctaaccg ttaaataatc tatatgaaag aggaaaatgt 1713
 gtctgtcgct tgatagagca ggcatcaacg ttgcataagt ttcttgaaga caaggaatgc 1773
 tacattatgt agtcccattc tggttcatgt attgtattgc taattatgac tagtattgat 1833
 tgttgttacc aagccgaatt ccagcacact ggcggccggt actagtggat ccgagctcgg 1893
 taccaaaaaa aaaaaaaaaa aaaaaa 1919

<210> 2
 <211> 507
 <212> PRT
 <213> Triticum aestivum

<400> 2

Met Val Asp Thr Leu Ser Glu Glu Asn Ile Ile Gly Tyr Phe Lys Asn
1 5 10 15

Lys Ser Ile Leu Ile Thr Gly Ser Thr Gly Phe Leu Gly Lys Ile Leu
20 25 30

Val Glu Lys Ile Leu Arg Val Gln Pro Asp Val Lys Lys Ile Tyr Leu
35 40 45

Pro Val Arg Ala Val Asp Ala Ala Ala Ala Lys His Arg Val Glu Thr
50 55 60

Glu Val Val Gly Lys Glu Leu Phe Gly Leu Leu Arg Glu Lys His Gly
65 70 75 80

Gly Arg Phe Gln Ser Phe Ile Trp Glu Lys Ile Val Pro Leu Ala Gly
85 90 95

Asp Val Met Arg Glu Asp Phe Gly Val Asp Ser Glu Thr Leu Arg Glu
100 105 110

Leu Arg Val Thr Gln Glu Leu Asp Val Ile Val Asn Gly Ala Ala Thr
115 120 125

Thr Asn Phe Tyr Glu Arg Tyr Asp Val Ala Leu Asp Val Asn Val Met
130 135 140

5 / 27

Gly	Val	Lys	His	Met	Cys	Asn	Phe	Ala	Lys	Lys	Cys	Pro	Asn	Leu	Lys
145					150					155					160
Val	Leu	Leu	His	Val	Ser	Thr	Ala	Tyr	Val	Ala	Gly	Glu	Lys	Gln	Gly
				165					170					175	
Leu	Val	Gln	Glu	Arg	Pro	Phe	Lys	Asn	Gly	Glu	Thr	Leu	Leu	Glu	Gly
			180					185						190	
Thr	Arg	Leu	Asp	Ile	Asp	Thr	Glu	Leu	Lys	Leu	Ala	Lys	Asp	Leu	Lys
		195					200					205			
Lys	Gln	Leu	Glu	Ala	Asp	Val	Asp	Ser	Ser	Pro	Lys	Ala	Glu	Arg	Lys
	210					215					220				
Ala	Met	Lys	Asp	Leu	Gly	Leu	Thr	Arg	Ala	Arg	His	Phe	Arg	Trp	Pro
225					230					235					240
Asn	Thr	Tyr	Val	Phe	Thr	Lys	Ser	Met	Gly	Glu	Met	Val	Leu	Ser	Gln
				245					250					255	
Leu	Gln	Cys	Asp	Val	Pro	Val	Val	Ile	Val	Arg	Pro	Ser	Ile	Ile	Thr
			260					265					270		
Ser	Val	Gln	Asn	Asp	Pro	Leu	Pro	Gly	Trp	Ile	Glu	Gly	Thr	Arg	Thr
		275					280					285			
Ile	Asp	Thr	Ile	Val	Ile	Gly	Tyr	Ala	Lys	Gln	Asn	Leu	Thr	Tyr	Phe
	290					295					300				
Leu	Ala	Asp	Leu	Asn	Leu	Thr	Met	Asp	Val	Met	Pro	Gly	Asp	Met	Val
305					310					315					320
Val	Asn	Ala	Met	Met	Ala	Ala	Ile	Val	Ala	His	Ser	Ser	Ser	Ser	Leu
				325					330					335	
Glu	Lys	Thr	Lys	Ser	His	Pro	Lys	Gln	His	Ala	Pro	Ala	Val	Tyr	His
			340					345					350		

6/27

Val Ser Ser Ser Leu Arg Asn Pro Ala Pro Tyr Asn Val Leu His Glu
 355 360 365

Ala Gly Phe Arg Tyr Phe Thr Glu His Pro Arg Val Gly Pro Asp Gly
 370 375 380

Arg Thr Val Arg Thr His Lys Met Thr Phe Leu Ser Ser Met Ala Ser
 385 390 395 400

Phe His Leu Phe Met Met Leu Arg Tyr Arg Leu Leu Leu Glu Leu Leu
 405 410 415

His Leu Leu Ser Ile Leu Cys Cys Gly Leu Phe Gly Leu Asp Thr Leu
 420 425 430

Tyr His Asp Gln Ala Arg Lys Tyr Arg Phe Val Met His Leu Val Asp
 435 440 445

Leu Tyr Gly Pro Phe Ala Leu Phe Lys Gly Cys Phe Asp Asp Val Asn
 450 455 460

Leu Asn Lys Leu Arg Leu Ala Met Thr Ser Asn His Gly Ser Leu Phe
 465 470 475 480

Asn Phe Asp Pro Lys Thr Ile Asp Trp Asp Glu Tyr Phe Tyr Arg Val
 485 490 495

His Ile Pro Gly Val Ile Lys Tyr Met Leu Lys
 500 505

<210> 3
 <211> 1949
 <212> DNA
 <213> Triticum aestivum

<220>
 <221> CDS
 <222> (74) .. (1642)
 <223>

<400> 3

7/27

ctctcttctt cctccgtctc tcattctctc ctcccagagct tcatagctca cacgcacaca	60
aaaccaaggc aag atg gtg ggc acg ctg gat gag ggg aag atc gtc gac	109
Met Val Gly Thr Leu Asp Glu Gly Lys Ile Val Asp	
1 5 10	
tac ttc agg aac aag agc gtg ctc atc acc gga gcc acg gga ttc ctt	157
Tyr Phe Arg Asn Lys Ser Val Leu Ile Thr Gly Ala Thr Gly Phe Leu	
15 20 25	
ggc aag ata atg gtg gag aag atc ctg cgg gtc cag ccg gac gtg aag	205
Gly Lys Ile Met Val Glu Lys Ile Leu Arg Val Gln Pro Asp Val Lys	
30 35 40	
agg atc tac ctg ccg gtg cgg gcg gcc gac gcc gcg gcg gcg agg cgc	253
Arg Ile Tyr Leu Pro Val Arg Ala Ala Asp Ala Ala Ala Ala Arg Arg	
45 50 55 60	
cgg gtg gag acc gag gtg gtg ggg aag gag ctg ttc tgc gtg ctg cgg	301
Arg Val Glu Thr Glu Val Val Gly Lys Glu Leu Phe Cys Val Leu Arg	
65 70 75	
gag cgc cac ggc gcc ggg ttc gac gcc ttc gtc gcc gac aag gtg gtg	349
Glu Arg His Gly Ala Gly Phe Asp Ala Phe Val Ala Asp Lys Val Val	
80 85 90	
ggg ctg gcc ggc gac gtc atg cgc gag ggc ttc ggc gtc gac ccc gcc	397
Gly Leu Ala Gly Asp Val Met Arg Glu Gly Phe Gly Val Asp Pro Ala	
95 100 105	
acg ctg cgg gac ctc cgg ctc gcc gac gag ctc aac gtc atc gtc aac	445
Thr Leu Arg Asp Leu Arg Leu Ala Asp Glu Leu Asn Val Ile Val Asn	
110 115 120	
ggc gcc gcc acc acc aac ttc tac gaa agg tac gac gtg gcc ctg gac	493
Gly Ala Ala Thr Thr Asn Phe Tyr Glu Arg Tyr Asp Val Ala Leu Asp	
125 130 135 140	
gtg aac gtg gtg ggg gtg aag cac atg tgc gac ttc gcc cgg agg tgc	541
Val Asn Val Val Gly Val Lys His Met Cys Asp Phe Ala Arg Arg Cys	
145 150 155	
ccc aac ctc gag gtg ctc atg cac gtc tcc acg gcc tac gtc gcc ggc	589
Pro Asn Leu Glu Val Leu Met His Val Ser Thr Ala Tyr Val Ala Gly	
160 165 170	
gag aag cag ggg ctg gtt ccg gag agg ccg ttc agg gac ggc gag acg	637
Glu Lys Gln Gly Leu Val Pro Glu Arg Pro Phe Arg Asp Gly Glu Thr	
175 180 185	
ctg cgc gac gac ggc acc caa ctc gac atc gac gcc gag atg agg ctg	685
Leu Arg Asp Asp Gly Thr Gln Leu Asp Ile Asp Ala Glu Met Arg Leu	
190 195 200	

8/27

gcc aag gac ctc agg aag cag atg gag gcc gac gac gat gtg gac ccc Ala Lys Asp Leu Arg Lys Gln Met Glu Ala Asp Asp Asp Val Asp Pro 205 210 215 220	733
aag gcc cag agg aag gcc atg aag gac ctc ggc ctc acc agg gcc agg Lys Ala Gln Arg Lys Ala Met Lys Asp Leu Gly Leu Thr Arg Ala Arg 225 230 235	781
cac ttt ggg tgg ccc aac acg tac gtg ttc acc aaa tcc atg ggg gag His Phe Gly Trp Pro Asn Thr Tyr Val Phe Thr Lys Ser Met Gly Glu 240 245 250	829
atg atg ctg gcc cag atg atg cgc ggg ggc gac gtg ccc gtc gtc atc Met Met Leu Ala Gln Met Met Arg Gly Gly Asp Val Pro Val Val Ile 255 260 265	877
gtc cgg ccc agc atc atc acc agc gtc cag aac gac cca ctg cca gga Val Arg Pro Ser Ile Ile Thr Ser Val Gln Asn Asp Pro Leu Pro Gly 270 275 280	925
tgg atc gaa ggc acc agg acg atc gac gca atc ctg atc ggg tac gcg Trp Ile Glu Gly Thr Arg Thr Ile Asp Ala Ile Leu Ile Gly Tyr Ala 285 290 295 300	973
aag cag agc ctg tcg tgc ttc ctc gcc gac ctc gac cta acc atg gac Lys Gln Ser Leu Ser Cys Phe Leu Ala Asp Leu Asp Leu Thr Met Asp 305 310 315	1021
gtg atg ccc ggc gac atg gtg gtg aac gcg atg atg gcg gcc acg gtg Val Met Pro Gly Asp Met Val Val Asn Ala Met Met Ala Ala Thr Val 320 325 330	1069
gca cat gcc tcc tcc act cag aca tca gag cca gag aag aag ccg cct Ala His Ala Ser Ser Thr Gln Thr Ser Glu Pro Glu Lys Lys Pro Pro 335 340 345	1117
ccg cag cag caa cac cct cac tcg gtg ccg gca gcg cca acg gtg tac Pro Gln Gln Gln His Pro His Ser Val Pro Ala Ala Pro Thr Val Tyr 350 355 360	1165
cac gtg agc tcg tcg ctg cgg cac ccg gct ccg tac gcg gtg ttg tac His Val Ser Ser Ser Leu Arg His Pro Ala Pro Tyr Ala Val Leu Tyr 365 370 375 380	1213
cga acg ggg atc cgg tac ttc gag gag cac cca cgg gtg ggg cct gat Arg Thr Gly Ile Arg Tyr Phe Glu Glu His Pro Arg Val Gly Pro Asp 385 390 395	1261
ggc cgc ccc gtg cgc acc cgt aag gtg cgg ttc ctc ggc agc atc gcg Gly Arg Pro Val Arg Thr Arg Lys Val Arg Phe Leu Gly Ser Ile Ala 400 405 410	1309

9/27

```

gcg ttc cac cta ttc atg gtg ctc aag tac cgt gtc ccc ctt gaa ctc      1357
Ala Phe His Leu Phe Met Val Leu Lys Tyr Arg Val Pro Leu Glu Leu
      415                                420                                425

ctc cgc ctg ctc tcc atc ctc tgt tgc ggc ctc ttt ggc ctt gcc gcc      1405
Leu Arg Leu Leu Ser Ile Leu Cys Cys Gly Leu Phe Gly Leu Ala Ala
      430                                435                                440

ctc tac cac gac ctc gcc cgc aag tac agg ttc gtg atg cag ctg gtg      1453
Leu Tyr His Asp Leu Ala Arg Lys Tyr Arg Phe Val Met Gln Leu Val
      445                                450                                455                                460

gac ctg tac ggg ccc ttc tcg ctc ttc aag ggt tgc ttc gac gat gta      1501
Asp Leu Tyr Gly Pro Phe Ser Leu Phe Lys Gly Cys Phe Asp Asp Val
      465                                470                                475

aac ctc aac aag ctc agg ctc gcc atg gcc gac ggt gac cat gcc gat      1549
Asn Leu Asn Lys Leu Arg Leu Ala Met Ala Asp Gly Asp His Ala Asp
      480                                485                                490

tcc gca ttc aac ttt gac ccc aag acc att gac tgg gac gac tac ttc      1597
Ser Ala Phe Asn Phe Asp Pro Lys Thr Ile Asp Trp Asp Asp Tyr Phe
      495                                500                                505

ttc aag gtc cac atc cct ggt gtc atg aag tac gtc cac aag tga      1642
Phe Lys Val His Ile Pro Gly Val Met Lys Tyr Val His Lys
      510                                515                                520

tggttctgtgt gcgatctgct tctgcgtgct gagaaggaat ggaggaaatc aaattaatgg      1702

tagcgctagt gtgccttgct tgtgttggtgt aacctccttc ttcgttcctc gagtattatt      1762

ggttgagtat tgattgtatt gtcattggaa gttaaattaa ccagtgacta tgagtataac      1822

taagatgaaa ttacttgcac catggcgggt ctctaaaact aagatagtac aaggatccta      1882

tgaagtacat tgaaattact tagtactttt catggtacta tcataataca aaaaaaaaaa      1942
aaaaaaaaa                                                                1949

```

```

<210> 4
<211> 522
<212> PRT
<213> Triticum aestivum

```

```

<400> 4

```

```

Met Val Gly Thr Leu Asp Glu Gly Lys Ile Val Asp Tyr Phe Arg Asn
1          5          10          15

```

10/27

Lys	Ser	Val	Leu	Ile	Thr	Gly	Ala	Thr	Gly	Phe	Leu	Gly	Lys	Ile	Met	20	25	30	
Val	Glu	Lys	Ile	Leu	Arg	Val	Gln	Pro	Asp	Val	Lys	Arg	Ile	Tyr	Leu	35	40	45	
Pro	Val	Arg	Ala	Ala	Asp	Ala	Ala	Ala	Ala	Arg	Arg	Arg	Val	Glu	Thr	50	55	60	
Glu	Val	Val	Gly	Lys	Glu	Leu	Phe	Cys	Val	Leu	Arg	Glu	Arg	His	Gly	65	70	75	80
Ala	Gly	Phe	Asp	Ala	Phe	Val	Ala	Asp	Lys	Val	Val	Gly	Leu	Ala	Gly	85	90	95	
Asp	Val	Met	Arg	Glu	Gly	Phe	Gly	Val	Asp	Pro	Ala	Thr	Leu	Arg	Asp	100	105	110	
Leu	Arg	Leu	Ala	Asp	Glu	Leu	Asn	Val	Ile	Val	Asn	Gly	Ala	Ala	Thr	115	120	125	
Thr	Asn	Phe	Tyr	Glu	Arg	Tyr	Asp	Val	Ala	Leu	Asp	Val	Asn	Val	Val	130	135	140	
Gly	Val	Lys	His	Met	Cys	Asp	Phe	Ala	Arg	Arg	Cys	Pro	Asn	Leu	Glu	145	150	155	160
Val	Leu	Met	His	Val	Ser	Thr	Ala	Tyr	Val	Ala	Gly	Glu	Lys	Gln	Gly	165	170	175	
Leu	Val	Pro	Glu	Arg	Pro	Phe	Arg	Asp	Gly	Glu	Thr	Leu	Arg	Asp	Asp	180	185	190	
Gly	Thr	Gln	Leu	Asp	Ile	Asp	Ala	Glu	Met	Arg	Leu	Ala	Lys	Asp	Leu	195	200	205	
Arg	Lys	Gln	Met	Glu	Ala	Asp	Asp	Asp	Val	Asp	Pro	Lys	Ala	Gln	Arg	210	215	220	

11 / 27

Lys 225	Ala	Met	Lys	Asp	Leu 230	Gly	Leu	Thr	Arg	Ala 235	Arg	His	Phe	Gly	Trp 240
Pro	Asn	Thr	Tyr	Val 245	Phe	Thr	Lys	Ser	Met 250	Gly	Glu	Met	Met	Leu	Ala 255
Gln	Met	Met	Arg	Gly 260	Gly	Asp	Val	Pro 265	Val	Val	Ile	Val	Arg	Pro	Ser 270
Ile	Ile	Thr 275	Ser	Val	Gln	Asn	Asp 280	Pro	Leu	Pro	Gly	Trp 285	Ile	Glu	Gly
Thr 290	Arg	Thr	Ile	Asp	Ala	Ile 295	Leu	Ile	Gly	Tyr	Ala 300	Lys	Gln	Ser	Leu
Ser 305	Cys	Phe	Leu	Ala	Asp 310	Leu	Asp	Leu	Thr	Met 315	Asp	Val	Met	Pro	Gly 320
Asp	Met	Val	Val	Asn 325	Ala	Met	Met	Ala	Ala 330	Thr	Val	Ala	His	Ala	Ser 335
Ser	Thr	Gln	Thr 340	Ser	Glu	Pro	Glu	Lys 345	Lys	Pro	Pro	Pro	Gln 350	Gln	Gln
His	Pro	His 355	Ser	Val	Pro	Ala	Ala 360	Pro	Thr	Val	Tyr	His 365	Val	Ser	Ser
Ser 370	Leu	Arg	His	Pro	Ala	Pro 375	Tyr	Ala	Val	Leu	Tyr 380	Arg	Thr	Gly	Ile
Arg 385	Tyr	Phe	Glu	Glu	His 390	Pro	Arg	Val	Gly	Pro 395	Asp	Gly	Arg	Pro	Val 400
Arg	Thr	Arg	Lys	Val 405	Arg	Phe	Leu	Gly	Ser 410	Ile	Ala	Ala	Phe	His 415	Leu
Phe	Met	Val	Leu 420	Lys	Tyr	Arg	Val	Pro 425	Leu	Glu	Leu	Leu	Arg 430	Leu	Leu

12/27

Ser Ile Leu Cys Cys Gly Leu Phe Gly Leu Ala Ala Leu Tyr His Asp
 435 440 445

Leu Ala Arg Lys Tyr Arg Phe Val Met Gln Leu Val Asp Leu Tyr Gly
 450 455 460

Pro Phe Ser Leu Phe Lys Gly Cys Phe Asp Asp Val Asn Leu Asn Lys
 465 470 475 480

Leu Arg Leu Ala Met Ala Asp Gly Asp His Ala Asp Ser Ala Phe Asn
 485 490 495

Phe Asp Pro Lys Thr Ile Asp Trp Asp Asp Tyr Phe Phe Lys Val His
 500 505 510

Ile Pro Gly Val Met Lys Tyr Val His Lys
 515 520

<210> 5
 <211> 1949
 <212> DNA
 <213> *Triticum aestivum*

<220>
 <221> CDS
 <222> (94)..(1617)
 <223>

<400> 5
 ctctctcttc ctctctctct ctctctctcc ctgctctccc tgctctctct ctctctctct 60
 ctctctcttg cgcacacaag aaaaaaatc aag atg gtt gac aca ctg agt gaa 114
 Met Val Asp Thr Leu Ser Glu
 1 5
 gag aag atc att gga tac ttc aag aac aag agc atc ctc atc act gga 162
 Glu Lys Ile Ile Gly Tyr Phe Lys Asn Lys Ser Ile Leu Ile Thr Gly
 10 15 20
 tca aca ggc ttt ctt gga aag ata cta gtg gag aag ata ctg aga gtt 210
 Ser Thr Gly Phe Leu Gly Lys Ile Leu Val Glu Lys Ile Leu Arg Val
 25 30 35
 caa cct gat gta aag aag atc tat ctc ccg gtg cga gcg gtg gat gcc 258
 Gln Pro Asp Val Lys Lys Ile Tyr Leu Pro Val Arg Ala Val Asp Ala
 40 45 50 55

13 / 27

gcg gcg gcg aag gat cgg gtg gag act gag gtg gta ggg aag gag ttg	306
Ala Ala Ala Lys Asp Arg Val Glu Thr Glu Val Val Gly Lys Glu Leu	
60 65 70	
ttc ggg ctt ctg agg gag aag cac ggg gac tgg ttt caa tct ttc atc	354
Phe Gly Leu Leu Arg Glu Lys His Gly Asp Trp Phe Gln Ser Phe Ile	
75 80 85	
tgt gaa aag atc gtc cca ttg gcc gga gat gtg atg cgt gag gac ttt	402
Cys Glu Lys Ile Val Pro Leu Ala Gly Asp Val Met Arg Glu Asp Phe	
90 95 100	
ggc gtc gac agc gag acc ctg agg gag ctc cgg gtg acc cag gag ctc	450
Gly Val Asp Ser Glu Thr Leu Arg Glu Leu Arg Val Thr Gln Glu Leu	
105 110 115	
gat gtc atc gtt aat ggc gcc gcc acc acc aac ttc tac gaa agg tat	498
Asp Val Ile Val Asn Gly Ala Ala Thr Thr Asn Phe Tyr Glu Arg Tyr	
120 125 130 135	
gat gtg gcc ctg gac gtg aac gtg atg gga gtg aag cat atg tgc aac	546
Asp Val Ala Leu Asp Val Asn Val Met Gly Val Lys His Met Cys Asn	
140 145 150	
ttc gcc aag aag tgc ccc aat ctc aag gtg ctc ctc cat gtc tcc acg	594
Phe Ala Lys Lys Cys Pro Asn Leu Lys Val Leu Leu His Val Ser Thr	
155 160 165	
gct tat gtt gcg ggt gag aag caa gga ctc gtg caa gag aga cca ttc	642
Ala Tyr Val Ala Gly Glu Lys Gln Gly Leu Val Gln Glu Arg Pro Phe	
170 175 180	
aag aat ggc gag acg ctg ctc gag ggg acc cac ctc gat atc gac acc	690
Lys Asn Gly Glu Thr Leu Leu Glu Gly Thr His Leu Asp Ile Asp Thr	
185 190 195	
gag ctg aaa ctg gcc aag gac ctg aaa aag cag ctt gag gcc gac gcc	738
Glu Leu Lys Leu Ala Lys Asp Leu Lys Lys Gln Leu Glu Ala Asp Ala	
200 205 210 215	
gac tcg tcg ccc aag tcc caa agg aag gcc atg aag gac ctt ggc atc	786
Asp Ser Ser Pro Lys Ser Gln Arg Lys Ala Met Lys Asp Leu Gly Ile	
220 225 230	
acc agg gcc cgg cac ttc ggg tgg ccg aac aca tac gtg ttc acc aag	834
Thr Arg Ala Arg His Phe Gly Trp Pro Asn Thr Tyr Val Phe Thr Lys	
235 240 245	
tcg atg ggg gag atg gtg ctg ggc cag ttg aag tgt gat ctc cct gtt	882
Ser Met Gly Glu Met Val Leu Gly Gln Leu Lys Cys Asp Leu Pro Val	
250 255 260	

14 / 27

gtc atc gtc cgt ccc agc atc atc acc agt gtc cag aac gac cca ctg	930
Val Ile Val Arg Pro Ser Ile Ile Thr Ser Val Gln Asn Asp Pro Leu	
265 270 275	
ccc gga tgg atc gaa ggc acc agg acg atc gac acg atc gtg atc ggc	978
Pro Gly Trp Ile Glu Gly Thr Arg Thr Ile Asp Thr Ile Val Ile Gly	
280 285 290 295	
tat gcg aag cag aac ctg aca tac ttc ttg gcg gac ctc aac ctc acc	1026
Tyr Ala Lys Gln Asn Leu Thr Tyr Phe Leu Ala Asp Leu Asn Leu Thr	
300 305 310	
atg gat gtg atg ccg ggc gac atg gtg gtg aat gcg atg atg gct gcc	1074
Met Asp Val Met Pro Gly Asp Met Val Val Asn Ala Met Met Ala Ala	
315 320 325	
atc gtg gcg cac agc tcg tcc tta ttg gag aag aca cag tca cat ccc	1122
Ile Val Ala His Ser Ser Ser Leu Leu Glu Lys Thr Gln Ser His Pro	
330 335 340	
gag cca cac gca ccg gcg gtg tac cac gtg agc tcg tcg cgg cgt aac	1170
Glu Pro His Ala Pro Ala Val Tyr His Val Ser Ser Ser Arg Arg Asn	
345 350 355	
ccg gcg ccg tac aat gtg ctg cac gag gct ggg ttt cgg tac ttc acg	1218
Pro Ala Pro Tyr Asn Val Leu His Glu Ala Gly Phe Arg Tyr Phe Thr	
360 365 370 375	
gag cac cct cgg gtg ggc cct gac ggc cgc acg gtg cgc acc cat aag	1266
Glu His Pro Arg Val Gly Pro Asp Gly Arg Thr Val Arg Thr His Lys	
380 385 390	
atg aca ttc ctc agc agc atg gct tcc ttc cac ctc ttt atg atg ctc	1314
Met Thr Phe Leu Ser Ser Met Ala Ser Phe His Leu Phe Met Met Leu	
395 400 405	
agg tac cgc ctc ctc ctt gag ctc ctc cac ctg ctc tcc gtc ctc tgt	1362
Arg Tyr Arg Leu Leu Leu Glu Leu Leu His Leu Leu Ser Val Leu Cys	
410 415 420	
tgt ggc ctc ttc ggc ctc gac acc ctc tac cac gac caa gca cgc aag	1410
Cys Gly Leu Phe Gly Leu Asp Thr Leu Tyr His Asp Gln Ala Arg Lys	
425 430 435	
tac agg ttc gtg atg cac ctg gtg gat ctg tat ggg ccc ttc gcg ctg	1458
Tyr Arg Phe Val Met His Leu Val Asp Leu Tyr Gly Pro Phe Ala Leu	
440 445 450 455	
ttc aag ggc tgc ttc gat gac gtc aac cta aac aag ctc agg ctc gcc	1506
Phe Lys Gly Cys Phe Asp Asp Val Asn Leu Asn Lys Leu Arg Leu Ala	
460 465 470	

15 / 27

```

atg acc agc aac cat gga agc ctc ttt aat ttc gac ccc aag acc att 1554
Met Thr Ser Asn His Gly Ser Leu Phe Asn Phe Asp Pro Lys Thr Ile
          475                      480                      485

gac tgg gac gat tac ttc tac agc gtc cac atc ccc ggg gtc cta aag 1602
Asp Trp Asp Asp Tyr Phe Tyr Ser Val His Ile Pro Gly Val Leu Lys
          490                      495                      500

cac atg ctc aac tga aatatccatg caccgaaaat ttaggcgttg ccttaataat 1657
His Met Leu Asn
          505

taaataatcg tacgttgtaa gaaatatcct cccaaattgg gttctaaccg ttaaataatc 1717

tgtgtgaaag agggaaatgt gtctgtcgct tgatagtgca ggcatcaact tgcatatgct 1777

tcttgaagaa caaggaatgc tacatcatgt agtttcgttc tggctcgtgc tgtattgcca 1837

attatgacta gtattgggttg ataacaattt cttgttcagt cacatattgt accttgacta 1897

gtattgattg ttaattataa tttcttttca agagaaaaaa aaaaaaaaaa aa 1949

```

```

<210> 6
<211> 507
<212> PRT
<213> Triticum aestivum

```

```

<400> 6

```

```

Met Val Asp Thr Leu Ser Glu Glu Lys Ile Ile Gly Tyr Phe Lys Asn
1          5          10          15

```

```

Lys Ser Ile Leu Ile Thr Gly Ser Thr Gly Phe Leu Gly Lys Ile Leu
          20          25          30

```

```

Val Glu Lys Ile Leu Arg Val Gln Pro Asp Val Lys Lys Ile Tyr Leu
          35          40          45

```

```

Pro Val Arg Ala Val Asp Ala Ala Ala Ala Lys Asp Arg Val Glu Thr
          50          55          60

```

```

Glu Val Val Gly Lys Glu Leu Phe Gly Leu Leu Arg Glu Lys His Gly
65          70          75          80

```

```

Asp Trp Phe Gln Ser Phe Ile Cys Glu Lys Ile Val Pro Leu Ala Gly
          85          90          95

```

16/27

Asp	Val	Met	Arg	Glu	Asp	Phe	Gly	Val	Asp	Ser	Glu	Thr	Leu	Arg	Glu			
			100					105					110					
Leu	Arg	Val	Thr	Gln	Glu	Leu	Asp	Val	Ile	Val	Asn	Gly	Ala	Ala	Thr			
		115					120					125						
Thr	Asn	Phe	Tyr	Glu	Arg	Tyr	Asp	Val	Ala	Leu	Asp	Val	Asn	Val	Met			
	130					135					140							
Gly	Val	Lys	His	Met	Cys	Asn	Phe	Ala	Lys	Lys	Cys	Pro	Asn	Leu	Lys			
145					150					155					160			
Val	Leu	Leu	His	Val	Ser	Thr	Ala	Tyr	Val	Ala	Gly	Glu	Lys	Gln	Gly			
				165					170					175				
Leu	Val	Gln	Glu	Arg	Pro	Phe	Lys	Asn	Gly	Glu	Thr	Leu	Leu	Glu	Gly			
			180					185					190					
Thr	His	Leu	Asp	Ile	Asp	Thr	Glu	Leu	Lys	Leu	Ala	Lys	Asp	Leu	Lys			
		195					200					205						
Lys	Gln	Leu	Glu	Ala	Asp	Ala	Asp	Ser	Ser	Pro	Lys	Ser	Gln	Arg	Lys			
	210					215					220							
Ala	Met	Lys	Asp	Leu	Gly	Ile	Thr	Arg	Ala	Arg	His	Phe	Gly	Trp	Pro			
225					230					235					240			
Asn	Thr	Tyr	Val	Phe	Thr	Lys	Ser	Met	Gly	Glu	Met	Val	Leu	Gly	Gln			
				245					250					255				
Leu	Lys	Cys	Asp	Leu	Pro	Val	Val	Ile	Val	Arg	Pro	Ser	Ile	Ile	Thr			
			260					265					270					
Ser	Val	Gln	Asn	Asp	Pro	Leu	Pro	Gly	Trp	Ile	Glu	Gly	Thr	Arg	Thr			
		275					280					285						
Ile	Asp	Thr	Ile	Val	Ile	Gly	Tyr	Ala	Lys	Gln	Asn	Leu	Thr	Tyr	Phe			
	290					295					300							

17 / 27

Leu Ala Asp Leu Asn Leu Thr Met Asp Val Met Pro Gly Asp Met Val
 305 310 315 320

Val Asn Ala Met Met Ala Ala Ile Val Ala His Ser Ser Ser Leu Leu
 325 330 335

Glu Lys Thr Gln Ser His Pro Glu Pro His Ala Pro Ala Val Tyr His
 340 345 350

Val Ser Ser Ser Arg Arg Asn Pro Ala Pro Tyr Asn Val Leu His Glu
 355 360 365

Ala Gly Phe Arg Tyr Phe Thr Glu His Pro Arg Val Gly Pro Asp Gly
 370 375 380

Arg Thr Val Arg Thr His Lys Met Thr Phe Leu Ser Ser Met Ala Ser
 385 390 395 400

Phe His Leu Phe Met Met Leu Arg Tyr Arg Leu Leu Leu Glu Leu Leu
 405 410 415

His Leu Leu Ser Val Leu Cys Cys Gly Leu Phe Gly Leu Asp Thr Leu
 420 425 430

Tyr His Asp Gln Ala Arg Lys Tyr Arg Phe Val Met His Leu Val Asp
 435 440 445

Leu Tyr Gly Pro Phe Ala Leu Phe Lys Gly Cys Phe Asp Asp Val Asn
 450 455 460

Leu Asn Lys Leu Arg Leu Ala Met Thr Ser Asn His Gly Ser Leu Phe
 465 470 475 480

Asn Phe Asp Pro Lys Thr Ile Asp Trp Asp Asp Tyr Phe Tyr Ser Val
 485 490 495

His Ile Pro Gly Val Leu Lys His Met Leu Asn
 500 505

<210> 7

18 / 27

<211> 3392

<212> DNA

<213> *Triticum aestivum*

<400> 7

cctctttaag	tcggtcaggt	acgtagcaaa	taaaccaaac	aagcaggaca	tgtttgaaaa	60
tcactgttct	aactttaata	atgcacacaa	ggaattgaag	gcttgataaa	tcctgtgcta	120
gtaccacca	tccgactgtc	ggttttttaag	acatggtaga	gtagagtact	aacataacga	180
tcttcaagat	attgtgtcca	agttctttgc	cacaggtttg	tcgtattgag	ccaaaatata	240
ccactgaatt	atgtgcta	at	agtgttaatta	ttgggtcaatt	ttttgttcta	300
acgagagaaa						360
aatacgctat	gcgttttgaa	gggatggagt	acgatgatac	tactctattg	tactccacgg	360
atcaattgta	ggagtaccct	ctaatttcta	atgggtataac	tttgatgaca	tacgatttgt	420
attacgttgg	ttgaattgta	catgggcctt	ctataaagcg	aaaaagaaag	gaatactgag	480
cttctctagt	tttctataam	ccagagttag	acgtcctact	ggacaactca	ttcctaagtg	540
tgtagcaata	atactgagta	ctaccgggtg	tccacttgag	ctattaattt	gatggctagc	600
aacgtactgc	aacccaata	ataaccctgt	ttgaaaatct	agagtacctc	tacgcgttat	660
tttgactgca	tgtgattcct	ctcatgatca	ccaggctccct	tgktttgggt	tggacagcgc	720
ataaatgttg	cgctcaagtg	cctcaaatta	agactcccct	ctcctaccta	caacacaaaa	780
gacctttttt	tcagcaaaag	agggcgccac	cccctccgat	ttcatataat	gaaaccaact	840
ggttcaactg	ccaacaaaac	aacaaaaaca	aaacgaaaca	ccttcatggc	ccgtgtataa	900
atattgtgta	catgaccctc	tttccactca	cccacacaaa	tcatctcctc	tctctctctc	960
tctctctctc	tctctcgcac	gcacgcacgc	acgcacacaa	gaaaaaaatc	aagatgggtg	1020
acacactgag	tgaagagaac	atcattggat	acttcaagaa	caagagcatc	ctcatcactg	1080
gatcaacagg	ctttcttgga	aagagtatgc	atgcatgcat	aatgtttatg	cacacatatt	1140
acgcatgcat	atactttttt	ttggcaaatt	ctagaagttt	tttgcgtaaa	gttcatatgt	1200
gctgtcctca	tctgttaatt	agttcactgt	tggtgtgcgt	atatgcagta	ctgggtggaga	1260
agatactgag	agttcaacct	gatgtgaaga	agatctacct	cccgggtgcga	gcgggtggatg	1320
ccgcggcggc	aaagcatcgg	gtggagactg	aggtagtgtt	gtccatcatg	gttcttcatg	1380
atctataccc	tttaccctc	tcatgattct	tttgatccct	tttcttcaga	gttagcgctg	1440

actaatttct	tttctatatg	cgcaggtggt	agggaaggag	ttgttcgggc	ttctgaggga	1500
gaagcacggg	ggcaggtttc	aatctttcat	ctgggaaaag	atcgtcccat	tggccggaga	1560
cgtgatgcgc	gaggacttcg	gcgtcgacag	cgagaccctg	agggagctcc	gggtgaccca	1620
ggagctcgat	gtcatcgta	atggcgccgc	caccaccaac	ttctacgaaa	ggtgcgtcat	1680
tgtcaactga	ttgttttgtc	caagaaagga	aaaatcagca	gagaacttga	gtaggtgcag	1740
caagagtacg	tacgcagtga	atgggtctcag	aagaacactt	gtgtgcgtgc	aggtatgatg	1800
tggctctaga	cgtgaacgtg	atgggagtga	agcatatgtg	caacttcgcc	aagaagtgcc	1860
ccaatctcaa	ggtgctcctc	catgtctcca	cgggtacgta	caccaactct	acaggtaaaa	1920
acaaaaataa	agttcttgga	tgttaattat	aatacaccta	gatttgattt	acaaatgaag	1980
ttaataaatt	catatatgag	ttgggtgcagc	ttacgtggcg	ggtgagaagc	aagggctggt	2040
gcaagagaga	ccattcaaga	atggcgagac	gctgctcgag	gggacccgcc	tcgacatcga	2100
cactgagctg	aaactggcca	aggacctgaa	aaagcagctt	gaggccgacg	ttgattcgtc	2160
gccaaggcc	gaaaggaagg	ccatgaagga	tcttggcctt	accagggccc	ggcacttcag	2220
gtggccaaac	acatacgtgt	tcaccaagtc	gatgggggag	atgggtgctaa	gccagttgca	2280
gtgtgatgtc	cccgttgta	tcgtccgtcc	cagcatcatc	acaagtgtcc	agaacgaccc	2340
actgcccgga	tggatcgaag	gcaccagggt	cattatatgt	ttcttgctct	ttctctgcct	2400
ccaaatttag	agtgcaatcg	tcttactctg	ttgcaaattgc	caaaagaagt	aaaatatgat	2460
atttgttcaa	tgtaaaaatg	taaattgcag	gacgatcgac	acgatcgtga	tcggctatgc	2520
gaagcagaac	ctgacatact	tcttggccga	cctcaacctc	accatggatg	tggttaagcaa	2580
cgttgtaacta	tgcattgcagt	taagatatat	tccaggcaat	ggttgggtgt	cagtccagtc	2640
caggaatccg	tacagtaaga	tgaatttcga	cgacgatggg	gaaagcaatc	gtttgggttg	2700
gtatatgttg	ctgtagatgc	cgggcgacat	ggtggtgaat	gcgatgatgg	cggcaatagt	2760
ggcacacagc	tcgtcctcat	tggagaagac	aaagtcacat	ccaagcaac	atgcaccggc	2820
ggtgtaccac	gtgagctcgt	cgctgcgtaa	tccggcacca	tacaatgtgc	ttcatgaggc	2880
tgggtttcgg	tacttcacgg	agcaccctcg	cgtgggccct	gacggtcgca	ccgtgcgtac	2940
ccataagatg	acattcctca	gcagcatggc	ttccttccac	ctatttatga	tgctcaggta	3000
ccgcctcctc	ctcgagctcc	tccacctgct	ctccatcctc	tgctgcggcc	tcttcggcct	3060

20 / 27

cgacaccctc taccacgacc aagcacgcaa gtacagggtta gttagttggt tgaaatcttg 3120
tgcggttgta tcttcttgat ggctcccaca taattaagat gacacgactt ttattgttggt 3180
attgttatag gttcgtgatg cacctgggtgg atctgtacgg gccctttgcg ctgttcaagg 3240
gggtgcttcga tgacgtcaac ctaaacaagc tcagggtcgc catgaccagc aaccatggta 3300
gcctcttcaa ttctgaccog aagaccattg attgggacga gtacttctac aggggtccaca 3360
tccccgggggt cataaagtac atgctcaagt ga 3392

<210> 8
<211> 5848
<212> DNA
<213> *Triticum aestivum*

<400> 8
ctgaccaaac atctcccatc aggaaccatg ttactattta attttgcattg ttctttgctt 60
cgtgctgttt gtcaaggaca cgctagcttc tgcattatac agacaggctg gaaatctgta 120
aataatgtgt tgggtgaatct ttggagtatt tttctaattg tgatcagtac ttgttgacct 180
aggtggcgac taactttgga caggggcaag caagcaaaag cagagacagc tagcttctgc 240
attatcagaa aatagcctgt gttgggtgaat cttcagagtg ctctactcc cccgtcccc 300
caaatgttca gtacttggtg acctactgga gctggacgac ccgctctgaa agttttactt 360
gtccacactt gtctggccaa aatccccaat gaatcagaat ataggctgaa aaatgagtgg 420
gtcgtttttg gagctcggcc tccacggagg ccgaaaaaaaa actcaaaatt ctaatttctc 480
agtttaaaaa aaaatgaaaa aaattgtaca agtaaacaag gttgtgatgt gtatgtgtgt 540
aaaatttcag acaaaaatac ttgaaatgc gatctgagca aaaaaaaca aattcataga 600
cttttgagaa tgaatagtgc aagctactaa aacgcatgag attctttttg cgtgtagcac 660
tcattttaat gtatttcgtc ctgaaaatta caaacttggt cattatgtct ataagtatat 720
gggtggattta aaaaaaaact gaaacatgca aatttgaatt ttgatcattt caaaatcggc 780
ctccatggag catttttgct gaaaaatcta aaagaaaacg cagtccaacc ttagtaatta 840
cccatgttgg tgaatccaga gtgtttacga taaatatagg acctctttag ctagcgaacg 900
atggcatttg cgaatgttat ttatggcaaa tccttcagaa cacacatgcc aatttcttca 960
taaacacatg gcaattctgg caaatatttt gctgtgaaaa ttgttggtgct tgcttcaagg 1020

aattgccatg	ttcccgcaat	ataaaatgcc	atgaaaaacg	ttcattttgt	catatcccca	1080
gcgaacgtcc	gccgagtagc	attaccgtaa	atataggggg	tgtttggttc	agaagttcta	1140
ggactttttc	tagtcccagg	gactaatcaa	aaaagactct	ctaatagagt	ctttttctag	1200
tccttataga	aaaagtccct	cccgtttggt	tcctagggac	tttttttagt	ctctaggact	1260
aaaaagtccc	tgaaccaaac	accccatatt	agtagtaccg	caaaaagtag	taagtactac	1320
agtattggtg	gatctttaat	gagttaatta	acctagaatg	gctcctgctc	ctccggtttc	1380
actggaaaag	caggttgtgg	ttgggaagca	caagcgtggt	gccgttgcta	attacgtgca	1440
ctcaatagcc	agccagccac	cctcaacacc	tcgctggccg	gccctgcgcg	tgtataaata	1500
ctgggtgccg	gagcacacaa	gcctcacaca	ccaagcacac	ctctcttctt	cctccgtctc	1560
tcattctctc	ctcccgagct	tcatagctca	cacgcacaca	aaaccaaggc	aagatggtgg	1620
gcacgctgga	tgaggggaag	atcgctcgact	acttcaggaa	caagagcgtg	ctcatcaccg	1680
gagccacggg	attccttggc	aagagtaagt	gagcatgatg	aggatataat	atatatgcat	1740
gccttgctat	tttctgaaga	acagttcatg	catccatgct	gagagtcctt	ggtggtgcat	1800
tgtgtgctca	ctcttcgacc	tgtgcagtaa	tggtggagaa	gacctgcggg	gtccagccgg	1860
acgtgaagag	gatctacctg	ccggtgcggg	cggccgacgc	cgcggcggcg	aggcgccggg	1920
tggagaccga	ggtgcgtgcc	tctctgcccg	tccatcatgc	tccagcatga	tggtgatcct	1980
tgcaccccct	ccatcaatct	cgctctgaac	actcctctgt	tctagagtaa	tactagtagt	2040
agacaaagga	tgcatgccgg	cagcatgggt	ctcatgctgc	aaggaacaca	tgcgagcttt	2100
ctctcagaca	gagaagcccc	caagctgctc	ccactgtgcc	ccttggtgtc	cttcattccg	2160
tttcatattc	actactcttt	ttttcattga	taatcaatac	tgtaatacta	gtaatattat	2220
gtggcgcatc	ggaacaaaac	atgcatagat	atcacagaac	cataaatagt	ccagctgccc	2280
gctgctatat	cagtgatgca	aacatgaaca	tggttagaat	agttatctta	taggcataaa	2340
ccggcattag	tcaggactag	gtgcacactg	ttgagtctca	tcctgccga	acattcaggc	2400
aggtcacatg	ccattttttc	ttctctttct	gaggttgaga	gtcacatgcc	atactctgca	2460
tgagaggtgg	ccatgaataa	tctcagctgt	acaccgctca	ttcttctcta	ctactccctc	2520
cgttcctaaa	tatgtctttt	taaagattcc	aacaagtgac	tacatacaga	ataaaatgaa	2580

tgaatctata	ttctaaaata	tgtctacata	catccatatg	ttatagtcca	tttaaaatgt	2640
ctaaaaaac	ttatathtag	gaacggaggg	agtagatact	agttaaagtc	acaaatatct	2700
tgccagcttt	tggatatgcca	ggattaattt	gaacgagaaa	ccagatacta	gtaggcgtat	2760
ggttgacgaa	atthttcaaca	ttttcattta	cgcccgatcc	tggaaaccgg	gaaccttggg	2820
aaaaccggcc	gcaggatgac	aattaaaccg	gcggcgaagt	agcgcagctg	tgtgtgtatc	2880
cctaggagtc	gtataggagt	tatggtgtgg	gtaggcgggt	aggtttggtc	aggtcgactc	2940
gattcacaag	ttttcaatgg	cctctgccgc	atgcagtgca	caactgggtac	tgcaccacca	3000
cctagctagc	tagccggcca	gtgtgcagcc	tgcattgcatg	catgcatgca	tggctggctg	3060
gctgctgctg	ggaaatgacg	agcgcagggc	accatctggc	actggcaccg	gcaccactaa	3120
ctacagcttc	atcaacttga	gaattcagaa	cccagagttt	cttgctcatat	gtgtatagag	3180
tggaccgcaa	gtctgcaatg	atgtgttggt	acctgattgc	atcaacaatg	aacacatata	3240
actgcaatta	tgtttttggtt	agtagattct	agtatatgtc	aaaagaaaaa	aagtgtagtt	3300
attgagtggg	gccaaggaga	tccagagcag	agacaatggg	cagtatgctt	ggctcctccac	3360
cctactccta	ctccatctag	taaaagccgg	gaaagaaacc	cttccagcac	gcctggcccc	3420
tcttcatttc	ttcatatgca	ttcactgatc	cttcattggt	cggcatgggc	aattttacagc	3480
tgcagacagt	tgcacgggtc	ggttgctcgtg	cgctgcagaa	gcaattttata	gtttttacca	3540
aaagttgcct	gagacactca	ccatgttggg	tgggtggatcc	acagtgcgtg	catgcatgta	3600
tcaaacgcac	attgcagggt	aagaattggg	gtaaatcaag	cacgtctatt	gatcaaacta	3660
atthttatcga	gaccggcgtg	gttcgcagggt	ggtgggggaag	gagctgttct	gcgtgctgcg	3720
ggagcgcacc	ggcgccgggt	tcgacgcctt	cgctcgccgac	aagggtgggtg	ggctggccgg	3780
cgacgtcatg	cgcgagggct	tcggcgctcga	ccccgccacg	ctgcgggacc	tccggctcgc	3840
cgacgagctc	aacgtcatcg	tcaacggcgc	cgccaccacc	aacttctacg	aaagggtgctg	3900
tgtactattg	actgttttaa	ttacttctgt	cagagagaaa	attcagcggg	agagaacgtg	3960
catggctgca	ccttcttctc	cagagtagtg	agctcatcaa	tggcctctgc	aactaacttt	4020
ggaatggacg	tgtgcgtgct	caggtacgac	gtggccctgg	acgtgaacgt	ggtggggggtg	4080
aagcacatgt	gcgacttcgc	ccggagggtgc	cccaacctcg	aggtgctcat	gcacgtctcc	4140
acggcctacg	tcgccggcga	gaagcagggg	ctgggttccgg	agaggccgtt	cagggacggc	4200

gagacgctgc gcgacgacgg caccacaactc gacatcgacg ccgagatgag gctggccaag 4260
gacctcagga agcagatgga ggccgacgac gatgtggacc ccaaggcca gaggaaggcc 4320
atgaaggacc tcggcctcac caggtttagca atcctatatt tttccaaagt ttctcctctt 4380
tcctgtgaaa ttcaagttca aaaaacagag ggttttatatt tgtgaaactt ggaactgaaa 4440
ttcaatatat ttttttgtaa ttgtgtgtgc agggccaggc actttgggtg gccaacacg 4500
tacgtgttca ccaaattccat gggggagatg atgctggccc agatgatgcg cgggggacgac 4560
gtgcccgtcg tcatcggtccg gcccgacatc atcaccagcg tccagaacga cccactgcca 4620
ggatggatcg aaggcaccag gtcggttcaa actctttggg tctaaatatg aatgccaagt 4680
ttcaaattca aattctaaca ccgaaatgaa aaatgcagga cgatcgacgc aatcctgatc 4740
gggtacgca agcagagcct gtcgtgcttc ctgcccagac tcgacctaac catggacgtg 4800
gtaattgctt cactcgttat tagttcagca aatgcagagc tgctagtgtt actgtcttct 4860
tgcaaacgtg ctgcagtaat gagtaataag tatactagta cagtgtgtga ccatgctgca 4920
gtaagttgca tctgaagcgt ctgtgccaac caggccaagt ggctgctgca gtgcatcagc 4980
caaccttaga tgaacttggc tgcgtgcggt tactatctga atcttgctg ttacagcaag 5040
gttacatatg ctgctactgg attatcaagt tctaaccctaa atgcgctacc atgggtgtctg 5100
cagatgcccg gcgacatggg ggtgaacgcg atgatggcgg ccacgggtggc acatgcctcc 5160
tccactcaga catcagagcc agagaagaag ccgcctccgc agcagcaaca ccctcactcg 5220
gtgccggcag cgccaacggg gtaccacgtg agctcgctgc tgcggcaccg ggctccgtac 5280
gcggtgttgt accgaacggg gatccgggtac ttcgaggagc acccacgggt ggggcctgat 5340
ggccgccccg tgcgcaccgg taagggtgcgg ttctcggca gcatcgcggc gttccacctt 5400
ttcatgggtg tcaagtaccg tgtccccctt gaactcctcc gcctgctctc catcctctgt 5460
tgcggcctct ttggccttgc cgccctctac cacgacctcg cccgcaagta cagggtacatt 5520
cttcttccgg aattagttga cgctcaaacg aattatctag agggagcaca gtttaaacac 5580
ttttgcggtt gtaccgtgtt gatgttatac cattggctta agaacatgca ttttgtgtag 5640
gttcgtgatg cagctgggtg acctgtacgg gcccttctcg ctcttcaagg gttgcttcga 5700
cgatgtaaac ctcaacaagc tcaggctcgc catggccgac ggtgacctg ccgattccgc 5760

24 / 27

attcaacttt gacccaaga ccattgactg ggacgactac ttcttcaagg tccacatccc 5820
tggtgtcatg aagtacgtcc acaagtga 5848

<210> 9
<211> 2800
<212> DNA
<213> *Triticum aestivum*

<400> 9
actccgacac aatattttga ctgcatgtga ttctctttat gatcactgag tctttgtttc 60
ggttggacag cgcataaatg ttgcgctcaa gtgcctcaaa ttaagacacc cctcttctac 120
ctacaacacg aaagaccctt ttttcttagc aaaaatttca tacaatgaaa ccaactgggtt 180
caactgccaa aacaacagac tccaagacaa aacaccttga tggcccgtgt ataaatattg 240
tgtacaggag cctctttcca ctacactaca caaatcatct ccttcttctt ctctctctct 300
ctctccctgc tctccctgct ctctctctct ctctctctct ctcttgcgca cacaagaaaa 360
aaaatcaaga tggttgacac actgagtga gagaagatca ttggatactt caagaacaag 420
agcatcctca tcaactggatc aacaggcttt cttggaaaga gtatgcatgc atgcacgcat 480
aatgtttata caaacatatt acgcatgcat atactatttg ttgcaaattc tagaagtttt 540
ttttttgcgt caagttgata tgtgttggtgc tcatctgtta attagttcta ctgttggtgc 600
atatgtgcag tactagtgga gaagatactg agagttcaac ctgatgtaaa gaagatctat 660
ctcccgggtgc gagcgggtgga tgccgcggcg gcgaaggatc ggggtggagac tgaggtagtg 720
ttgtccatca tggttcttca tgatctagac ctctactgct ctcatgattc ttttgatccc 780
tttgcttgag tgttggtact gaataattta ttttctatgt gccaggtgg tagggaagga 840
gttggtcggg cttctgaggg agaagcacgg ggactggttt caatctttca tctgtgaaaa 900
gatcgtccca ttggccggag atgtgatgcg tgaggacttt ggcgtcgaca gcgagaccct 960
gagggagctc cgggtgaccc aggagctcga tgtcatcggt aatggcgccg ccaccaccaa 1020
cttctacgaa aggtgcgtcg tcgtcaactg attgttttgt ccaagaaagg aaaaaaatc 1080
agcagagaac ttgagtaggt gcagcaagag tacggagtgg aattgtgtgc gtgcaggtat 1140
gatgtggccc tggacgtgaa cgtgatggga gtgaagcata tgtgcaactt cgccaagaag 1200
tgccccaatc tcaagggtgct cctccatgtc tccacgggta cgtacaccaa ctctacaaat 1260

25 / 27

taccatcatg gactatgaac ttggatgctt ctggggaaaa caaaaatgaa gttcttggat	1320
gtaattaaag tacacctaga tttgatttac aaatcaagtt aatgaattca tacatgagtt	1380
ggtgcagctt atgttgcggg tgagaagcaa ggactcgtgc aagagagacc attcaagaat	1440
ggcgagacgc tgctcgaggg gacccacctc gatatcgaca ccgagctgaa actggccaag	1500
gacctgaaaa agcagcttga ggccgacgcc gactcgtcgc ccaagtccca aaggaaggcc	1560
atgaaggacc ttggcatcac cagggcccgg cacttcgggt ggccgaacac atacgtgttc	1620
accaagtcga tgggggagat ggtgctgggc cagttgaagt gtgatctccc tgttgctcatc	1680
gtcgtccca gcatcatcac cagtgtccag aacgaccac tgcccggatg gatcgaaggc	1740
accaggttcg ttatatgctt ctctttcctt tcctgcctc taaatttaaa gtgcaatcgt	1800
tttaatctgt tgcaaatgca agtaaataaa ggatgtttgt tcaatgtaaa aatgtaaatt	1860
gcaggacgat cgacacgac gtgatcggct atgcgaagca gaacctgaca tacttcttgg	1920
cggacctcaa cctcaccatg gatgtggtaa gcaacgttgc actatgcatg cagttaatta	1980
acatatattc caggcatgca atggttgggt gtcagtccag gaatccatac agtaagatat	2040
ggatttcaac gatggcgggtg aatgcaatcg tgtggttggg tatatgttgg tgcagatgcc	2100
gggcgacatg gtggtgaatg cgatgatggc tgccatcgtg gcgcacagct cgtccttatt	2160
ggagaagaca cagtcacatc ccgagccaca cgcaccggcg gtgtaccacg tgagctcgtc	2220
gcggcgtaac ccggcgccgt acaatgtgct gcacgaggct gggtttcgggt acttcacgga	2280
gcacctcgg gtgggccctg acggccgcac ggtgcgcacc cataagatga cattcctcag	2340
cagcatggct tccttcacc tctttatgat gctcaggtag cgcctcctcc ttgagctcct	2400
ccacctgctc tccgtcctct gttgtggcct cttcggcctc gacacctct accacgacca	2460
agcacgcaag tacaggttag tcggtttaaa tcttttgcg atggcatttt tgataacaag	2520
tatttccgga cggagggagt atcttcttga tggcgcgga tatatgatga cacggccttt	2580
attgttatat tgttgtaggt tcgtgatgca cctggtggat ctgtatgggc ccttcgcgct	2640
gttcaagggc tgcttcgatg acgtcaacct aaacaagctc aggctcgcca tgaccagcaa	2700
ccatggaagc ctctttaatt tcgaccccaa gaccattgac tgggacgatt acttctacag	2760
cgtccacatc cccgggggtcc taaagcacat gctcaactga	2800

26 / 27

<210> 10
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> OL2707 primer

<400> 10
gactacgtcg tccaaggccg

20

<210> 11
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> OL2708 primer

<400> 11
gtcgaactgc ttgagcagcg c

21

<210> 12
<211> 19
<212> DNA
<213> OL2881 primer

<400> 12
gcagaacctg acatacttc

19

<210> 13
<211> 18
<212> DNA
<213> Artificial sequence

<220>
<223> OL2885 primer

<400> 13
gaggcggtac ctgagcat

18

<210> 14
<211> 18
<212> DNA
<213> OL2884 primer

<400> 14
ttcgcatagc cgatcacg

18

27 / 27

<210> 15
<211> 18
<212> DNA
<213> Artificial sequence

<220>
<223> OL2883 primer

<400> 15
aatgccggcc ctggtaag

18

<210> 16
<211> 18
<212> DNA
<213> Artificial sequence

<220>
<223> OL2880 primer

<400> 16
caggtggcca aacacata

18

Applicant's or agent's file reference 46450-PT	International application No
---	------------------------------

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**
(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>19</u> , line S <u>4 - 14</u> .	
B. IDENTIFICATION OF DEPOSIT pAMW170 Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution International Depositary Authority of Canada	
Address of depositary institution (including postal code and country) Bureau of Microbiology, Health Canada 1015 Arlington Street Winnipeg, Manitoba R3E 3R2 CANADA	
Date of deposit June 7, 2001	Accession Number IDAC 070601-1
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
Applicant hereby informs the International Bureau that the microorganism should be made available to third parties only by the issue of a sample to an expert nominated by the requestor.	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) All states which allow for this provision under national/regional law.	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer <i>Carole Millan</i>	Authorized officer

Applicant's or agent's file reference 46450-PT	International application No
--	------------------------------

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>19</u> , lines <u>4 - 14</u> .	
B. IDENTIFICATION OF DEPOSIT pAMW133 Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution International Depositary Authority of Canada	
Address of depositary institution (including postal code and country) Bureau of Microbiology, Health Canada 1015 Arlington Street Winnipeg, Manitoba R3E 3R2 CANADA	
Date of deposit June 7, 2001	Accession Number IDAC 070601-2
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
Applicant hereby informs the International Bureau that the microorganism should be made available to third parties only by the issue of a sample to an expert nominated by the requestor.	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) All states which allow for this provision under national/regional law.	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

<p>For receiving Office use only</p> <p><input checked="" type="checkbox"/> This sheet was received with the international application</p> <p>Authorized officer <i>Carole Millau</i></p>	<p>For International Bureau use only</p> <p><input type="checkbox"/> This sheet was received by the International Bureau on:</p> <p>Authorized officer</p>
---	--